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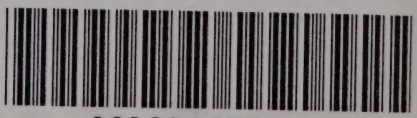

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OUTLINES

OF

PRACTICAL HISTOLOGY

OUTLINES

OF

PRACTICAL HISTOLOGY

BY

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SECOND EDITION

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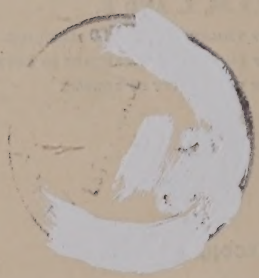

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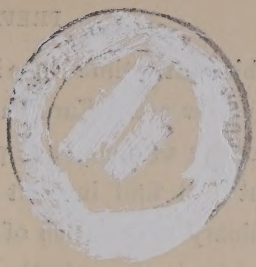

PREFACE

TO

THE SECOND EDITION.

No subject more than Practical Histology requires for its successful pursuit that guidance which *oral* instruction alone can give. If the beginner attempts to study it without a teacher, he is baffled in learning how to use the microscope, how to manipulate the tissues, and how to apprehend rightly the phenomena that come under his observation. Much valuable time is thus lost, and the final result is generally failure and disappointment.

These Outlines are therefore primarily intended to assist beginners who are working under the guidance of a teacher in a laboratory furnished with the usual appliances, together with a full set of those microscopical specimens that cannot be readily made. They are at the same time, however, designed to assist those who may already have learned the rudiments of

the subject at a time when its details were not taught as they now are. Care has been taken to render all instructions as definite as possible, and the advanced student will find in Part I. such directions for the preliminary preparation of the tissues as will enable him to work through the Histological Demonstrations in Part III. without embarrassment. The paragraphs in small type are especially intended for the advanced student.

These Outlines are essentially the Notes of the course of Practical Histology given by me in King's College, London, and in the University of Edinburgh. That course is conducted on the principle that, inasmuch as the time at the disposal of most students of medicine is very limited, the only practicable method of teaching large numbers is what may be termed the *regimental* system, whereby the students all work at the same moment, at one subject, and are addressed collectively. In this way classes, under thirty in number, can be conducted through the leading points of Practical Histology in about thirty-six lessons, extending from an hour to an hour and a half. As it is impossible in this short time to prepare every desirable microscopical specimen, special demonstrations are made by the teacher in order that the *introduction* to the subject may be as comprehensive as possible. The special

working out of many complicated processes cannot be attempted in a general course of this sort, but must be done by the advanced student working in the laboratory for several hours daily. The exceptionally high magnifying powers sometimes mentioned in the following pages, may be used by him, but in the case of the beginner it is understood that they are intended to be used for special demonstration by his teacher.

In the course of the *demonstrations* (Part III.), the student is desired to read the general considerations regarding *methods*, in Part IV.; and when he finds no statement regarding the preservation of a specimen, it is to be inferred that it is needless to preserve it.

In this edition, the book has been considerably extended by an account of the microscope, and fuller descriptions of the tissues. All *figures* of the latter have been omitted, however, for they are to be found in the systematic text books already in the hands of everyone. Their repetition here would therefore have entailed needless addition to the expense of the work. It is hoped that the demonstrations of the tissues, if made as directed, will supply the place of pictures, and that the descriptions will, together with hints from the teacher, prove a sufficient guide to them.

The blank paper at the end is intended for drawings and notes.

The author is glad of the indication of the usefulness of the book, afforded by the rapidity with which the first edition was exhausted.

UNIVERSITY OF EDINBURGH,
September 1876.

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ABBREVIATIONS.

H. means *high* power; a combination of lenses such as the $\frac{1}{6}$ inch objective of an English microscope, with an eye-piece of medium power, or the No. 7 objective and No. 3 eye-piece of Hartnack's microscope—with the tube of the microscope drawn out—magnifying about 300 diameters linear.

L. means *low* power; a combination of lenses such as the 1 inch objective of an English microscope, with an eye-piece of medium power, or the No. 3 objective and No. 3 eye-piece of Hartnack's microscope, magnifying about 50 diameters.

V. S., L. S., and T. S., respectively mean vertical, longitudinal, and transverse section.

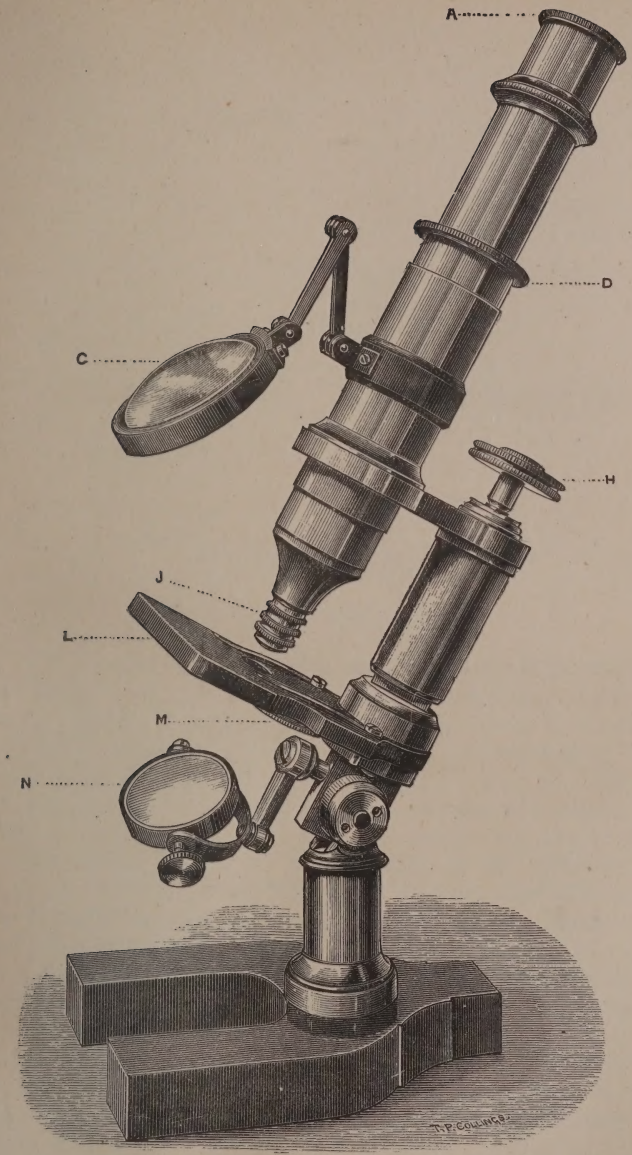

FIG. 1.—Hartnack's No. IIIA Microscope. *A*, Eyepiece. *D*, Coarse adjustment. *C*, Condenser. *H*, Fine adjustment. *Y*, Objective. *L*, Stage. *M*, Diaphragm. *N*, Mirror.

OUTLINES

OF

PRACTICAL HISTOLOGY.

PART I.

INTRODUCTION.

APPARATUS REQUIRED.

1. THE student who is entering upon the study of Practical Histology must be provided with a compound microscope capable of giving a low magnifying power of about 50 diameters, and a high power of about 300 diameters; a thin-bladed razor of good steel (§ 294); a pair of strong needles in handles (§ 290); scalpels, forceps, and scissors, such as are found in an ordinary dissecting case, the bent scissors shown in Fig. 55 are, however, to be preferred; three or four camel-hair brushes of medium size; five or six dozen ground glass slides, 3 × 1 inch; about a hundred *extra thin* circular cover-glasses, $\frac{3}{4}$ inch in diameter; a box to hold about fifty preparations. The student will thus be enabled to preserve the principal specimens made during the course of study indicated in the following pages.

2. He should also be provided with the following set of the ordinary reagents and mounting fluids. These may be conveniently placed in one-ounce bottles, with ordinary corks (Fig. 2).*

* Corks of vulcanised indiarubber are not suitable.

1. ¾ per cent Salt Solution (§ 269)
2. Magenta Solution (§ 324 *a*)
3. Picrocarmine Solution (§ 322)
4. Logwood Solution (§ 323)
5. Dilute Acetic Acid (§ 313)
6. Dilute Alcohol (§ 284)
7. Glycerine
8. Farrants' Solution (§ 349)
9. Dammar mounting fluid (§ 347*c*)

} In bottles, with a glass rod fixed in the cork.

10. Clove Oil. In a bottle, with a small *sable* hair brush fixed to a stick (Fig. 2).

11. Gold Size Cement
12. White Zinc Cement

} In wide-mouthed bottles, with camel-hair brushes.

It is convenient to place these bottles in a wooden framework similar to that used for test-tubes, and to cover the whole with a paper or glass shade, to protect it from dust.

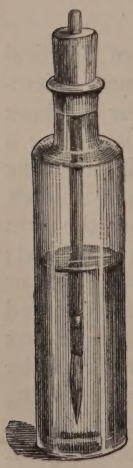

FIG. 2.—Corked reagent bottle.

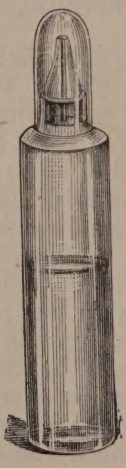

FIG. 3.—Capillary reagent bottle.

The advanced student will find it advantageous to place the first eight of the above-mentioned reagents in the bottles with capillary stoppers (Fig. 3). In these the reagent is completely protected from the entrance of dust by a glass cap. These bottles, however, though excellent for very fine work, are not to be recommended for ordinary purposes, because of the serious length of time required to obtain a drop of the reagent — the bottle requiring to be inverted, and held in the hand until its heat expands the air and expels the fluid.

3. The advanced student who may be desirous of working through the following course of instruction privately—without the resources of a laboratory—requires, in addition to the foregoing apparatus and reagents, a magnifying power of about 800 or 1000 diam.; a stage and an eye-piece micrometer (§§ 59, 60); a freezing microtome (§ 302); a camera lucida (§ 58, *a* or *b*); a lamp with a chimney of pale blue glass for night work (§ 51); a warm stage (§ 261); a small and a large injection syringe (Fig. 60), and perhaps a pressure bottle injection apparatus (Fig. 61); a turn-table (Fig. 63). A polarising apparatus may be added, but other accessories are of very little use. Various other reagents are required, but these need not be particularised here.

PREPARATION OF TISSUES PREVIOUS TO THEIR EXAMINATION.*

4. The following fluids require to be prepared:—

I. $\frac{3}{4}$ *per cent Salt Solution.* Dissolve 7·5 grammes of dried ordinary NaCl in 1000CC distilled water.

II. *One per cent Chromic Acid Solution.* Dissolve 10 grammes chromic acid in 1000CC water. This can be readily diluted when required.

III. *Chromic Acid and Spirit Solution.* Dissolve 1 gramme chromic acid in 20CC water, and *slowly add it* to 180CC rectified spirit.

IV. *Chromic and Bichromate Solution.* Dissolve 1 gramme chromic acid, 2 grammes potassium bichromate in 1200CC water.

V. *Chromic and Nitric Fluid.* Chromic acid 1 gramme, water 200CC, then add 2CC nitric acid.

VI. *Müller's Fluid.* Dissolve 25 grammes potass. bichrom. and 10 grammes sodium sulphate in 1000CC water.

VII. *One per cent Bichromate Solution.* Dissolve 10 grammes potass. bichromate in 1000CC water.

* This section is designed to assist the advanced student when he undertakes histological work in private.

5. Most of the requisite tissues and organs may be obtained from the cat and guinea-pig. Feed the cat, and an hour or so afterwards place it in a bag; drop chloroform over its nose until insensibility is produced. Open the chest by a linear incision through the sternum; speedily open the right ventricle, and allow the animal to bleed to death.

6. Divide the trachea immediately below the cricoid cartilage, and inject it with $\frac{1}{4}$ per cent chromic acid fluid; tie it to prevent the escape of the fluid, and place the distended lungs in the same fluid, and cover them with cotton wool. Change the fluid at the end of eighteen hours. Allow them to remain in this fluid for a month, then cut into small pieces and transfer to rectified spirit* until required.

7. Open by a linear incision the stomach, small and large intestine, and wash their inner surface with $\frac{3}{4}$ per cent salt solution. Place the tongue, divided transversely into a number of pieces, and a portion of the small intestine in chromic and bichromate fluid (Solution 4). Change the fluid at the end of eighteen hours; and at the end of a fortnight transfer them to rectified spirit until required. Place the tongue, divided transversely into five or six pieces, together with a portion of the stomach and large intestine, in $\frac{1}{4}$ per cent chromic acid solution. Change the fluid at the end of eighteen hours; and at the end of a month transfer them to rectified spirit till required.

8. Open the bladder, wash it with salt solution, and place it in a 1 per cent solution of bichromate of potash (Solution 7) for two or three days.

9. Remove the kidneys; divide one transversely, the other longitudinally, and place them in Müller's fluid. Change the fluid at the end of eighteen hours; and at the end of four weeks transfer them to rectified spirit until required. They will be ready after having been a fortnight in the spirit. It is also advantageous to have the kidneys

* In this edition rectified spirit has been substituted for methylated spirit in the preparation of the tissues, for undoubtedly the tissue elements are better prepared in it. (Its sp. gr. is 0·838.)

of a rabbit prepared as follows :—Divide the kidneys longitudinally; place them for a fortnight in a 1 per cent solution of chromic acid, then in rectified spirit till required.

10. Cut half of the liver into small pieces, and place them in Müller's fluid. Change the fluid at the end of eighteen hours; and at the end of four weeks transfer them to rectified spirit till required.

11. Place the spleen, uterus, some thin muscles from the limbs or abdomen, in $\frac{1}{4}$ per cent chromic acid. Change the fluid at the end of eighteen hours; and at the end of a month transfer them to rectified spirit.

12. Through several points in the tunica albuginea of testis of cat, inject a 1 per cent solution of osmic acid, and then place it in rectified spirit; and finally—a day or two before making sections—in absolute alcohol.

12A. Place the ovaries of cat or dog in Müller's fluid. Change the fluid after the first day, and after three weeks transfer to a mixture of equal parts of absolute alcohol and water for thirty-six or forty-eight hours, then in absolute alcohol for a day, after which they will be ready for section.

13. Remove both eyes. Divide them transversely behind the crystalline lens. Remove the vitreous. Place the posterior halves in the chromic and spirit (Solution 3). Change the fluid at the end of eighteen hours. Transfer to rectified spirit at the end of ten days. Place the crystalline lens in chromic acid and spirit for two weeks, and then in rectified spirit. Place the cornea in $\frac{1}{4}$ per cent chromic acid for a month, and then in rectified spirit.

14. Cautiously open the cranial and spinal cavities. Remove brain and spinal cord, and strip off arachnoid. Partially divide spinal cord into pieces half an inch long. Partially divide the brain transversely into a number of pieces. Place them in a *cool* place in rectified spirit for eighteen hours. Transfer the spinal cord to $\frac{1}{4}$ per cent chromic acid for six or seven weeks. Change the fluid at the end of eighteen hours. Prepare the sciatic nerve in the same manner. Place the brain in chromic and bichromate fluid. Change at the end of eighteen hours, and then once a week, until the brain is hard and tough. If it

be not tough and leathery at the end of six weeks, place it in $\frac{1}{6}$ per cent chromic acid for a fortnight, and then in rectified spirit. If the brain be placed in a too strong chromic acid solution, or if it be too long exposed to the action of even a moderately strong solution, it becomes friable and useless. Procure a portion of human cerebrum (*parietal lobe*), as fresh as possible, and treat it in the same manner as the cat's brain. A portion of human cerebellum may be prepared in the same manner. At the end of six or seven weeks transfer all the tissues to rectified spirit to complete the hardening. The brain and spinal cord should always be placed on cotton wool in their hardening fluid, in order that the fluid may reach every part equally.

15. Remove the muscles, but *not the periosteum*, from the bones of the upper or lower limb, and remove both from the lower jaw. Divide the jaw and the long bones transversely in two or three places, and put them into the chromic and nitric fluid (Solution 5). Change the fluid repeatedly until the bone is just sufficiently softened, and then transfer it to rectified spirit. If the softening is not completed in a month, double the quantity of nitric acid in the fluid, or place them simply in dilute nitric acid (2 per cent). The softening is most rapidly accomplished by suspending the bones in the fluid. For this purpose they may be placed in a muslin bag. The bone must not, however, remain in the softening fluid after all calcareous matter has been removed.

16. Place a piece of human scalp, skin from palmar surface of finger, in chromic and spirit fluid (Solution 3). Change the fluid at the end of eighteen hours. At the end of a month transfer to rectified spirit.

17. Remove the petrous portion of the temporal bone, open the tympanum, pull the stapes from the oval fenestra, and place the cochlea in chromic and spirit fluid. Change it at the end of eighteen hours, and again on the sixth or seventh day. But if a copious brown precipitate fall, change the fluid every third day. On the tenth or twelfth day transfer to chromic and nitric fluid. Change the fluid frequently until the bone is soft. Then place

it in rectified spirit. In the guinea-pig the cochlea forms a very evident projection into the tympanum, and is therefore very convenient for enabling the student to see how the cone is to be sliced when sections are to be made. Kill a young guinea-pig, sever the head from the body, disarticulate the lower jaw, open the tympanic bulla, remove the stapes, and prepare the cochlea as above. In a *young* animal the chromic and spirit fluid alone may suffice to soften the bone, and the chromic and nitric fluid may therefore not be required, or at any rate only for a short time.

It is very important to place all the above tissues in a *cool* place. A cellar is best in summer. Protoplasm rapidly undergoes change in even a medium temperature.

PART II.

THE MICROSCOPE.

FUNDAMENTAL PRINCIPLES.

18. **The Visual Angle.**—We form an opinion of the magnitude of an object from the size of the image which it produces on the retina. The lateral rays from any object intersect behind the crystalline lens. The size of the retinal image depends on the size of the visual angle —that is, the angle formed by the intersection of the rays

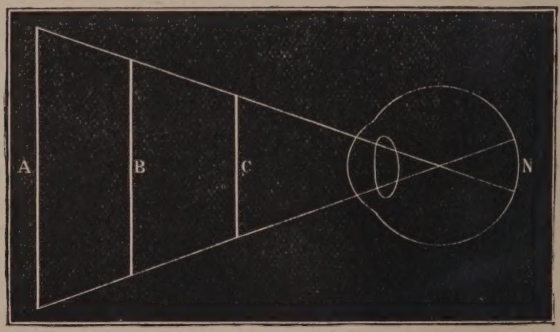

FIG. 4.—Visual angle.

from the extreme points of the object. All objects which have the same visual angle appear of the same size. Thus the objects A B C (Fig. 4), although actually of different sizes, have—when placed at proper relative distances from the eye—the same apparent size ; because the visual angle is the same for all, and the magnitude of the image on the retina (N) consequently the same.

Moreover, an object of the same actual size appears of different sizes at different distances from the eye,—the magnitude appearing less the greater the distance. This depends on the size of the visual angle being inversely as the distance of the object from the eye. Thus, if we look at a line in the position A (Fig. 5), it produces the

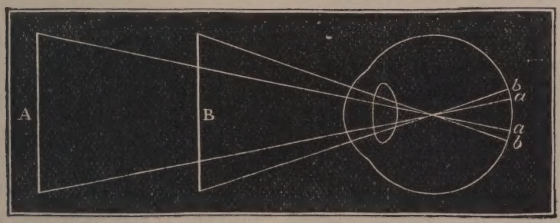

FIG. 5.—Visual angle.

image *a a* on the retina: in the position B it appears larger; because its image *b b* excites a greater retinal area; in the latter the visual angle is evidently greater than in the former.

19. **Theory of the Microscope.**—The eye can see the same object clearly when it is successively placed at various distances, because of an alteration of the curvature of the crystalline lens, whereby parallel rays from an object at a great distance, or divergent rays from a near object, may be successively focalised accurately upon the retina. The more minute the object, the nearer one brings it to the eye in order to increase the visual angle, and get as large an image as possible. But there is a limit to this. Within eight or ten inches from a normal eye of an adult, the object cannot be seen without an unpleasant effort to render the crystalline lens sufficiently convex to focalise the divergent rays on the retina; and within two inches or so the eye completely fails to accomplish the task; the image is therefore ill defined. But if a convex lens (Fig. 19) be interposed between the near object and the eye, it acts in the same sense as the crystalline lens, and assists it focussing the divergent rays, and thus the object apparently magnified can be clearly seen.

20. Refraction and Reflection of Light.—The action of a lens depends on the following principles. As long as a luminous ray travels in a uniform medium it pursues a straight line. If it pass at a right angle from one medium to another, it does not deviate from its course, however different the refractive powers of the two media may be. But if the ray traverse the plane separating two media of different refractive powers at any other than a right angle, it is bent. Thus, the ray R (Fig. 6), passing through air and striking the surface of a glass plate obliquely at B, is bent in two directions; a portion (r) is *reflected*, while the remainder is *refracted towards* the perpendicular (p). The refracted ray again strikes the plane between the two media obliquely at C; a portion (r') is reflected, while the remainder is refracted from the perpendicular as it re-enters the air. The emergent ray is of less intensity after traversing the glass, in consequence of its partial reflection at the two surfaces; there is therefore *a loss of light at every refraction*; a very important fact in microscopical optics.

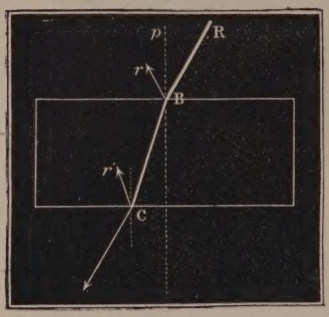

FIG. 6.—Refraction and reflection by parallel plate.

21. Index of Refraction.—The refractive power of any medium is expressed by its index of refraction. This is ascertained by measuring the amount of refraction which a ray undergoes in passing from air into another medium; thus, let r (Fig. 7) be a ray passing through air, incident upon water at i, and refracted in the direction r'. The sines of the angles of incidence $r\ i\ p$, and of refraction $r'\ i\ p'$, are obtained by describing a circle with the point

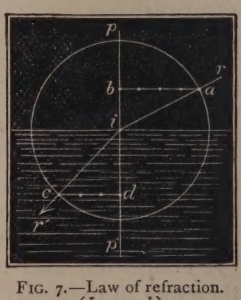

FIG. 7.—Law of refraction. (*Lommel.*)

i as the centre, and drawing lines from the perpendicular at right angles with it to the points at which the circle intersects the incident and refracted rays. Thus *a b* and *c d* are the sines of the angles of incidence and refraction respectively. In the case of air and water the sines are as 4 : 3. The refractive index of air is so small, that for practical purposes its refractive power is discarded, and therefore $\frac{4}{3} = 1{\cdot}333$ the refractive index of water. The following table shows the indices of refraction for various substances, many of which are used in practical histology :—

INDICES OF REFRACTION.

Diamond . .	2·44
Flint glass . .	1·642
Crown glass . .	1·530
Canada balsam .	1·540
Clove oil . .	1·535
Oil of turpentine .	1·478
Glycerine . .	1·475
Alcohol . .	1·372
Aqueous humour .	1·337
Pure water . .	1·336
Air . . .	1·000294

As will afterwards be seen, such substances as Canada balsam, clove oil, turpentine, and glycerine, having a comparatively high refractive index, are constantly employed in histology, for the purpose of increasing the transparency of tissues.

22. **Lenses.**—If a ray pass obliquely through a medium with parallel faces (Fig. 6), the excident assumes the same direction as the incident ray, but, if the faces be not parallel, the excident ray assumes a new direction. Thus, let *r* (Fig. 8) be a ray impinging on a triangular prism at *a*, it bends towards the perpendicular *p*, and on emerging at *b*, bends from it. The excident ray *r'* thus takes up a direction entirely different from *r*. If two prisms be joined by their *bases* (Fig. 9), the rays A B, being refracted as indicated in Fig. 8, are rendered convergent and brought to

a focus (C). On the other hand, if the *angles* of the

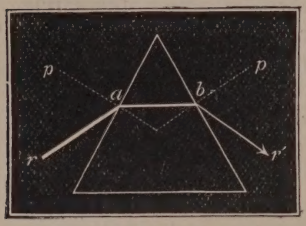

FIG. 8.—Refraction by prism.

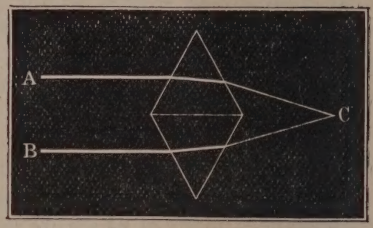

FIG. 9.—Convergence of rays by prisms.

prisms be turned towards each other (Fig. 10), the rays A B, are rendered divergent. The converging lenses A B and C in Fig. 11 act after the manner of the prisms in Fig. 9, while the diverging lenses D E and F (Fig. 11) act like the prisms arranged as in Fig. 10.

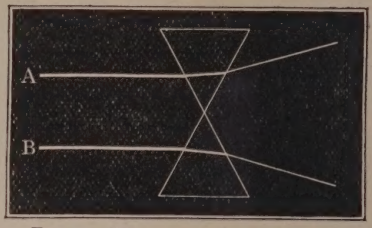

FIG. 10.—Divergence of rays by prisms.

FIG. 11.—Lenses.

23. **Focal Length.**—Take the simple case of a plano-convex lens (Fig. 12). It may be regarded as composed of two rectangular prisms joined by their bases, with the oblique surfaces curved so as to form the segment of a sphere whose radius is C S. The rays A R R all strike the plane surface of the lens at right angles, and therefore undergo no refraction until the spherical surface is reached. The ray A S passing through the centres of both surfaces of the lens is

termed the *axial ray*. Being perpendicular to both surfaces, it undergoes no refraction. The rays R S, on emerging from the lens, are bent from the perpendicular C S, and meet in the focus F. In a plano-convex lens the

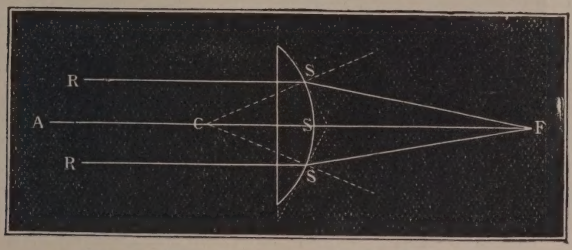

FIG. 12.—Effect of plano-convex lens. (*Hannover.*)

optical centre is placed at the point where the axial ray meets the convex surface; in a bi-convex lens it is placed within the lens, at its very centre, if the convexity of the surfaces be equal. The distance of the focal point F from the optical centre is the focal distance of the lens. In a plano-convex lens of crown glass the focal distance is almost exactly *twice* the radius of curvature (C S), while in a bi-convex lens of the same substance having equally curved surfaces, the focal length is very nearly the same as the radius of curvature. But the focal length depends not only upon the *degree of curvature* of the lens, it also depends on the *refractive power* of its material; thus, if the focal lengths of two lenses—the one of flint, the other of crown glass—of equal curvatures be compared, the flint lens is found to have a shorter focus than the other, because of its higher refractive power (§ 21).

24. **Principal Focus and Conjugate Foci.**—The *principal* focus of a convex lens (A, Fig. 13) is the point (F) to which rays that were *parallel* before entering the lens are brought. (The same is shown in Fig. 14, where P P are the parallel rays, and F the principal focus.) If the rays be divergent (D D, Fig. 14) before entering the lens, they are focalised outside the principal focus (D′); while, if they be convergent (C C), they are focalised within

it (C′). It is evident that if a cone of rays proceeded from a luminous point at F, the divergent pencil would be converted into a cylinder of parallel rays. If the luminous

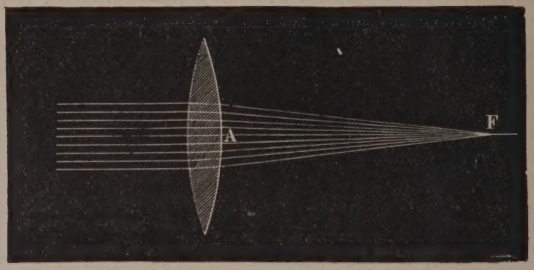

FIG. 13.—Principal focus. (*Deschanel.*)

point were placed within the principal focus—say at C′, the more divergent cone would not be rendered parallel by the lens, but would still continue to diverge, though to a smaller degree, after passing through it; but if the luminous pencil

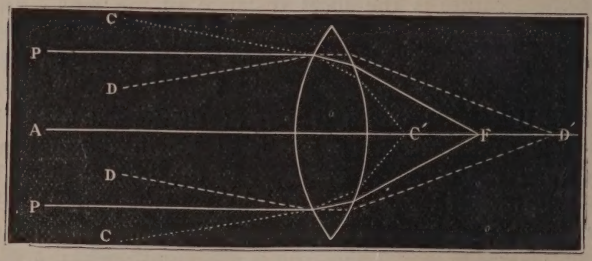

FIG. 14.—Principal focus (F) and secondary foci (C′ D′). (*Hannover.*)

proceeded from a point *outside the principal focus*, say at D′, the less divergent rays would be rendered convergent (D D) by the lens, and would come to a focus somewhere on the other side of it. In Fig. 15 a cone of rays is seen proceeding from a point S′, outside the principal focus F, and brought by a lens to a *real* focus at S. At S a real image of the point S′ would be produced. Conversely, if the rays proceeded from S, they would come to a *real* focus at S′, and if S were placed nearer the lens, but outside the

principal focus, S′ would recede from the lens. The points S and S′ are therefore termed *real conjugate foci;* that is,

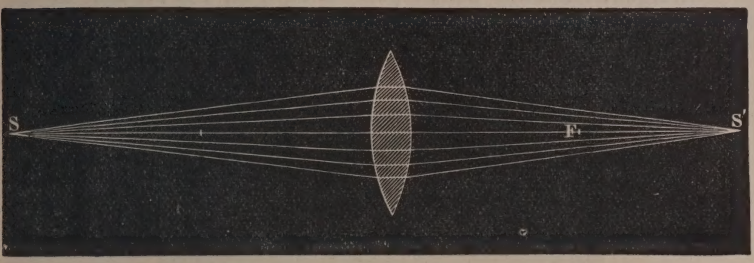

FIG. 15.—Conjugate foci, both real. (*Deschanel.*)

points so correlated that rays diverging from the one converge to the other. In Fig. 15 the luminous point is placed in the principal axis of the lens; if it were placed above or below the axis, the rays would be focalised on the opposite side of the axis (Fig. 17).

25. **Virtual Focus.**—Let Fig. 16 represent a luminous point T placed *within* the principal focus F of the lens A B, the rays are never brought to a focus, but are only rendered less divergent by the lens. The rays after traversing the lens proceed just as if they emanated from the point V, to which they would pass if prolonged backwards, without the intervention of the lens. To an observer a luminous object at T would appear to be at V. V would therefore be a *virtual* but *not* a *real* image of the object T, or, otherwise expressed, T is a real, and V a virtual focus.

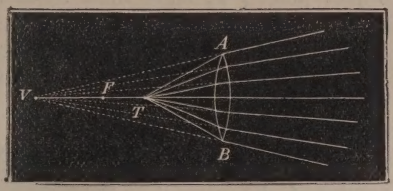

FIG. 16.—Real and virtual foci. (*Lommel.*)

26. **Real Image.**—If a luminous object be placed at *twice* the focal distance from a convex lens, a *real* image of the *same size* is produced at the same focal distance; but if the object (*a b*, Fig. 17) be placed at *less than twice* the

focal distance, but *outside the principal focal point* (F), a *real magnified image* of the object (A B) is produced. The divergent luminous pencil proceeding from *a* is brought to a real focus at A. The position of A is determined by the point at which the refracted ray *a g* intersects the secondary

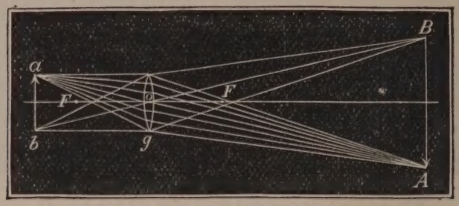

FIG. 17.—Production of a *real* magnified image. (*Lommel.*)

axial ray *a* O A :—that is, the ray passing from *a* through the optical centre of the lens. Luminous pencils, of course, proceed from every point of the object *a b*, and are focalised at corresponding points of A B ; these, however, are for the sake of clearness omitted from the figure.

Conversely, it will be readily seen that if A B were the object placed at *more than twice the focal distance*, a *real diminished* image would be produced at *a b*. The *real* images produced by a convex lens are always *inverted.*

27. **Virtual Image, with the Simple Microscope.**—If an object A B (Fig. 18) be placed *within* the focal distance of a convex lens, no *real* image can be produced, because the rays proceeding from A issue divergingly from the lens as if they came from the *virtual* point *a*. Although in this case the *lens cannot focalise the rays proceeding from* A B, *the crystalline* lens of *the eye of an observer can do this, and thereby produce a real* image of A B on the retina, provided that A B is placed only a little way within the focal distance of the

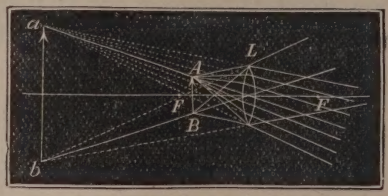

FIG. 18.—Virtual magnified image. (*Lommel.*)

lens (L). To him the rays from A would appear as if they proceeded from *a*. He would therefore see an enlarged *virtual* image (*a b*) of the object. *This is the principle of the simple microscope.* Fig. 19 explains the theory of enlargement by the simple microscope more clearly. Let A

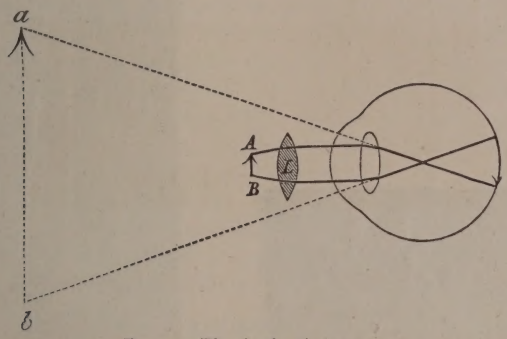

FIG. 19.—The simple microscope.

B be an object placed within but near the principal focus of the lens L, the rays from A B are rendered less divergent by the lens, and are focalised by the crystalline lens of the eye upon the retina. To the observer they appear as if they proceeded from an object in the position *a b* at the distance of ten inches from the eye.

The simple microscope is often used for dissection (§ 50) and other purposes, where a high magnifying power is not required.

28. **Compound Microscope.**—The compound microscope is always employed where high magnifying powers are needed. Its simplest form (Fig. 20) consists of two convex lenses, one—the objective (O) placed near the object, the other—the ocular (O′), placed next the eye. The object (*a b*) is placed slightly *beyond* the focal length of the objective O. A *real* magnified image is produced at *a′ b′ c′*, slightly within the focal distance of the ocular O′, by which it is again magnified, so that the observer sees the *virtual* magnified image A B C. The compound microscope is therefore really just a conjunction of two

simple microscopes. The complete form of the compound microscope (Fig. 21) has an objective (O) consisting of

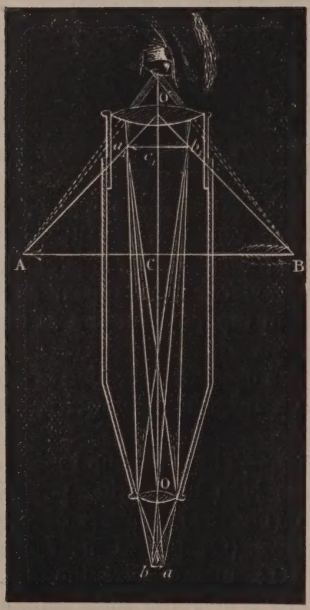

FIG. 20.—Compound microscope—*Simplest form.* (*Deschanel.*)

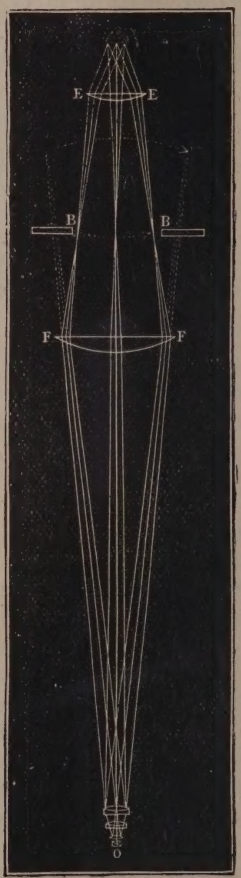

FIG. 21.—Compound microscope—*Complete form.* (*Carpenter.*)

several lenses superimposed, while the ocular is always composed of two converging lenses, the lower one, termed the field-glass (F F), collecting the rays from O. so as to

form at B B a real image of a dimension capable of being seen through the upper lens, termed the eye-glass, E E. A diaphragm or stop B B, consisting of a blackened metal disc with a hole in the centre, cuts off the extraneous rays, and thus gives sharpness to the image.

29. **The Faults of a Lens.**—There are two faults in every lens which must be overcome before a satisfactory image of an object can be produced. These faults are of trivial importance with very low magnifying powers, but in the case of high powers they become so serious, if not corrected, that the microscope is useless. The faults are :— 1. Spherical, 2. Chromatic aberration.

30. *Spherical aberration* is due to the manner in which rays are refracted by a spherical surface. The axial ray A (Fig. 22), striking the lens at a right angle, is not refracted. The rays C C around the axis are bent to a slight extent

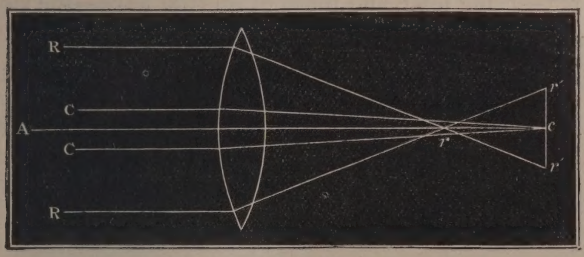

FIG. 22.—Spherical aberration.

and brought to a focus at *c*; the lateral rays R R, striking the lens much more obliquely, are focalised at *r*; consequently the image of an object is rendered indistinct, its margins *r' r'* are misty and ill-defined, owing to the lateral rays having already passed their focal point.

31. Spherical aberration is to a large extent remedied by intercepting the lateral rays with a diaphragm. This method is often had recourse to in simple microscopes, but it has the disadvantage of diminishing the aperture of the lens, and thereby causing a loss of light. In the eyepiece of the compound microscope, however, where the lenses are large, a diaphragm is used (B B, Fig. 21).

Other methods of preventing spherical aberration are also had recourse to; thus, in the *eyepiece* of the microscope, two lenses (E F, Fig. 31) are so arranged that the rays which are internal in passing through the one become external in passing through the other, so that the spherical fault of the one corrects that of the other. The manner in which spherical aberration is corrected in the objective would require an explanation entirely beyond the limits of this work. It must, therefore, be simply stated that it is by a combination of converging with diverging lenses, of such curvature and arranged at such distances, that the positive spherical aberration of the one set is neutralised by the negative spherical aberration of the other.

32. *Chromatic aberration* is due to the fact that the different coloured rays which compose white light are unequally refrangible; those of the longest wave length—the red—being least, and those of shortest wave—the violet

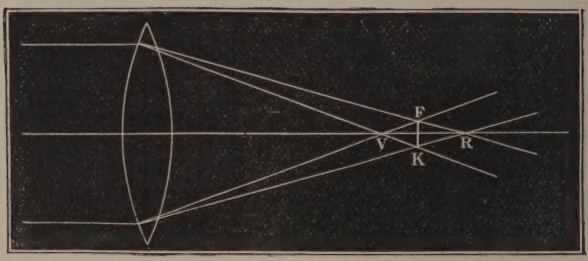

FIG. 23.—Chromatic aberration.

—being most easily bent. In passing through a lens, therefore, white light is *dispersed*, the violet rays are focalised (V, Fig. 23) nearer the lens than the red (R), while the foci of the other colours are intermediate between these. The image of a colourless object therefore appears coloured. The coloration is most marked at V and R, least so at F K; for in that plane there is an intersection of the red and violet rays.

33. To understand the manner in which chromatic aberration is remedied, it is necessary to carefully distinguish between the *deflection* and the *dispersion* of light.

The indices of refraction, already alluded to (§ 21), are really the indices of *deflection;* they indicate the amount of deflection that a ray of homogeneous light, *e.g.* pure red, would undergo when transmitted through various substances. The table of indices (§ 21) shows that in *flint* glass the *deflective* power is 1·642, while in *crown* glass it is 1·53. It would, however, be easy to have a flint prism that would give the same deflection as a crown prism; the former only requires to be made of a smaller angle than the latter; thus a prism of flint glass with a refracting angle of 52° would have the same deflective power as a 60° prism of crown glass; a ray of pure red light would be deflected to the same extent by both. But if a ray of white light were transmitted through each prism, *the spectrum produced by the flint would be nearly twice as long as that produced by the crown glass;* that is to say, the *dispersive* power of flint is nearly double (1·7 in exact figures) that of crown glass.

34. The construction of an achromatic lens then is essentially this. If we combine in opposite positions two prisms, one of crown glass of about 60° (*c*, Fig. 24), the other (*f*) of flint glass of about 30°, a ray (*r*) passing into the prism *c* is deflected and dispersed.

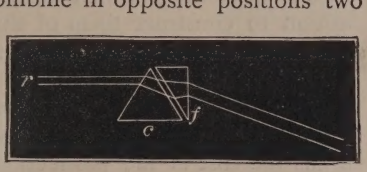

FIG. 24.—Achromatic prisms. (*Lommel.*)

On passing into *f* it is deflected and dispersed in an opposite direction. But, owing to the smaller angle of *f*, the outward (*upward* in the Fig.) deflection of the ray does not overcome its inward deflection in *c*, therefore, it emerges with a part of the deflection given to it in *c* still retained. But the *dispersive* power of flint being nearly double that of crown glass, the outward *dispersion* in *f*

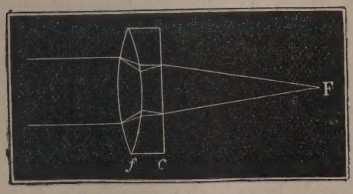

FIG. 25.—Achromatic lens.

is greater than the outward deflection; and so, by a careful adjustment of the angles of the prisms, the dispersive power of *f* can exactly reverse that of *c*, while the deflection is not overcome, hence the ray *r* emerges from *f* *deflected* but *not dispersed.* The system is *achromatic.* An achromatic object-glass may consist (Fig. 25) of a bi-convex lens of crown, and a plano-concave lens of flint glass cemented together with Canada balsam. The effect is the same as that of the prisms in Fig. 24. Luminous rays, whether travelling to or from the point F, leave the lens achromatic.

THE OPTICAL PARTS OF THE COMPOUND MICROSCOPE.

35. **The Objective.** — The value of a compound microscope depending principally on the quality of the objective, it is usually termed *the* lens. It consists of one or more compound lenses (O, Fig. 21). In objectives of medium power, *e.g.* $\frac{1}{6}$-inch focus, it has been generally the fashion to construct them of three compound plano-convex achromatic glasses. Recently, however, Mr. Wenham (*Proc. Roy. Soc.* vol. xxi. p. 111) has devised an objective, in which only one concave lens of dense flint glass is used to correct four convex lenses of crown glass. By this means the cost of the production of lenses of high quality has been much lessened.

36. The powers of continental lenses are usually distinguished by the numbers 1, 2, 3, and so on; in this country, their powers are indicated by their focal length. The lenses most useful for the beginner are the 1-inch and $\frac{1}{6}$-inch;* giving, with a suitable ocular, a magnifying power of about 50 and 300 diameters. Much higher powers in addition to these are required by the advanced student, such as $\frac{1}{10}$, $\frac{1}{16}$, and $\frac{1}{20}$ or $\frac{1}{25}$. A $\frac{1}{50}$ has been constructed, but for ordinary purposes, the $\frac{1}{25}$ is as high a power as need be employed. The lenses made by Hartnack and by Zeiss, on the Continent, are commendable on account of

* These have a power similar to objectives 3 and 7 of Hartnack's microscope.

their comparatively small cost and good quality. The lenses made in England, especially those by Ross, R. and J. Beck, Powell and Lealand, are of great excellence: Wenham's $\frac{1}{7}$ made by Ross, the new $\frac{1}{8}$ by Powell and Lealand, and the $\frac{1}{10}$ by R. and J. Beck, are all lenses of such beauty as has, in the opinion of competent authorities, not been equalled elsewhere.

37. **Angle of Aperture.**—The angle of aperture of an objective is the angle formed between the most external rays that can penetrate its *entire system* of lenses from a luminous point placed in the focus; thus, in Fig. 26, rays are seen diverging from a luminous point in the focus *f*: some of them (*a a'*) enter the first or *front* lens, too near its periphery to get through into the second. The rays *b b'* are the most peripheral rays that are capable of passing through all the lenses of the objective, and therefore the angle between *b* and *b'* is the angle of aperture: from this it appears that the *actual* aperture of a lens is a very different thing from its *angular* aperture. There has been much discussion regarding the relative merits of lenses of small and of large angular aperture. With a large angle more of the oblique rays are admitted, and therefore for the *resolution* (definition) of very fine lines, such as those on diatom scales, and the rods and cones of the retina, the larger angle is preferable; but the more the angle is increased, the greater is the loss of *penetrating power;* by *penetrating* power is meant the power of seeing with tolerable distinctness points lying a little above and below the exact focus. This is a matter of very great importance in studying the structure of tissues and organs. It may be readily understood from Fig. 26 that the smaller the angle, the more can the objective be raised or lowered without throwing the object much out of focus. At the same time, however, lenses of large angle give a more brilliantly illuminated field, and are on that account more agreeable to

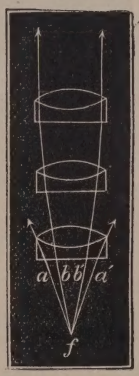

FIG. 26.—Angle of aperture of the objective. (*Dippel.*)

work with. It may be safely stated that for ordinary histological purposes, lenses of "small," or rather of *medium*, are preferable to those of "large" angle. In such a lens as $\frac{1}{6}$ inch, 70° is considered a "small," and 130° a "large" angle.

38. **Adjustment of the Objective for covered and uncovered Objects.** — If rays pass from a luminous point (S, Fig. 27) to the eye obliquely through a parallel glass plate, there is an apparent displacement of the point to the position S′. The distortion of an oar when dipped in the water is due to a similar cause. If, therefore, a plate of glass be interposed between the lens and an object, the latter is distorted, and the amount of distortion *increases with the thickness of the glass.* Thus, in Fig. 28, we have rays diverging from the point *a* and passing through a cover-glass *c*. The ray *r*, close to the perpendicular *p*, would appear to an observer to proceed from an object very nearly in the position of *a*,—the ray *r′* from an object at *a′*, and *r″* from an object at *a″*. The single luminous point *a* would thus be *spread out* and rendered indistinct; and the *more so the thicker the cover-glass; hence the importance of employing thin cover-glass.* It is evident from Fig. 28 that the divergence of the rays is increased by the cover-glass; hence, if the objective be ad-

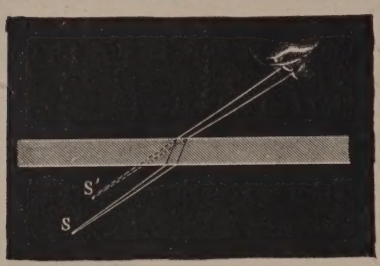

FIG. 27.—Apparent displacement of a luminous point by a parallel plate. (*Deschanel.*)

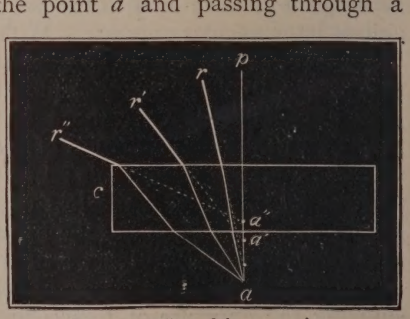

FIG. 28.—Effect of the cover-glass.

justed so as to clearly define an object without a cover-glass, it will fail to do so if it have a cover. Mr. Ross first pointed this out, and made the front lens of the objective movable, so that the observer might adjust it for covered or uncovered objects (Fig. 29). The front lens is fixed in the case *a*—the inner lenses, in *b*, *e*, and *e'*, are two lines engraved on the outer case *a*, and *d* a line on a portion of the inner case projecting through a slit in *a*. For a *covered* object the lenses are approximated by a screw until the line *e* is opposite *d*, while, for an uncovered object, they are separated until the line *e* is opposite *d*. For ordinary lenses, however, this adjustment is unnecessary, for one really scarcely ever looks at an uncovered object, and, moreover, the adjustment is liable to shift and thereby throw things into confusion. For very high powers, however, it is essential, because cover-glasses vary a good deal in thickness, and if the cover be not of the very thickness with which the optician corrected the lens, definition is impaired. In that case, therefore, it is important to have an adjusting screw on the lens.

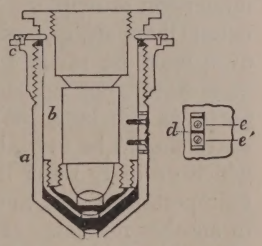

FIG. 29.—Objective with adjustment for covered and uncovered objects.

39. **Dry and Immersion Lenses.**—With a dry lens, the rays pass through air between it and the cover-glass. With an immersion lens, some fluid, such as water or glycerine, is interposed. Very high powers are now almost always constructed on the immersion system, for one of its great advantages is the conservation of light. Fig. 30 explains this; in A the oblique ray *r* is seen passing from the cover-glass past the lens. In B it passes from the cover-glass into water, the refractive index of which being

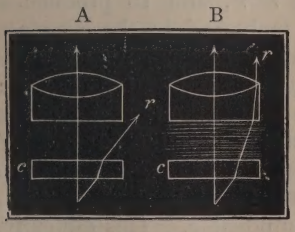

FIG. 30.—A. Dry. B. Immersion lens.

higher than that of air, the ray is not so much deflected from the perpendicular at the upper surface of *c* in B, as in A, and therefore it enters the lens. In the case of very high powers, the full illumination of the object is a most essential consideration; hence the importance of the immersion principle. For the same reason that water is better than air, glycerine is better than water; care, however, must be taken to employ *pure* glycerine. Glycerine is, however, not so convenient as water, and for ordinary purposes the latter is all that is required; only *distilled* water should be used, however, for the film upon the lens, which ordinary water leaves when it dries, is by no means unimportant. The immersion system is also advantageous in another respect. If Fig. 28 be compared with B, Fig. 30, it will be evident that, by diminishing the obliquity of the rays, the water under the immersion lens must contribute to the better *definition* of the object. The immersion system should, however, be employed only for very high powers, it being inconvenient in doing ordinary work. By Ross and some other opticians, lenses are now made that may be used either dry or immersed by altering an adjustment.

40. **The Eye-Piece or Ocular.**—The eye-piece commonly employed is that invented by Huyghens. It consists of two plano-convex lenses placed as in Fig. 31 (E F). The rays from the objective would, if uninterrupted, form an image at *v v* and *r r*. With the field-glass (F) they are focalised at *v' v'* and *r' r'*, and so produce a smaller image capable of being embraced by the eye-glass (E). Both lenses produce chromatic and spherical aberration, but these are reduced to a minimum as follows:—If the objective were perfectly achromatic, the light would be decomposed by F, and the violet rays focalised *nearer* to F than the red rays (Fig. 23). The focal length of E being, as in the case of F, *shorter* for the violet than for the red, an achromatic image would be impossible. This difficulty, however, is overcome by *over-correcting* chromatic aberration in the objective, so that the coloured light issuing from it is focalised by the converging lens F in a manner the

reverse of that indicated in Fig. 23; that is to say, the violet rays V V are focalised at a *greater* distance from F than the red rays R R. The focus of E being, of course, shorter for the violet than for the red, these different rays are focussed by E on the same points of the retina, and thus an image, colourless or nearly so, is seen. The manner in which spherical aberration in the eye-piece is prevented by adjusting its lenses, so that the spherical fault of the one corrects that of the other, has been already explained (§ 31).

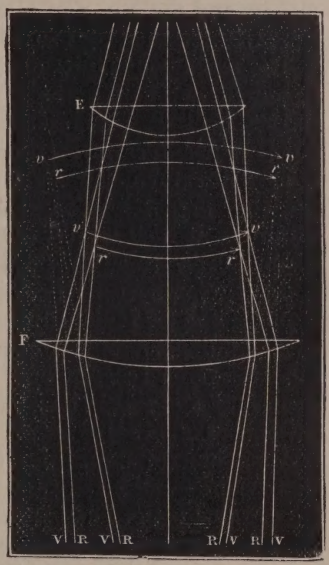

FIG. 31.—The eye-piece of Huyghens. (*Dippel, slightly altered.*)

With most microscopes eye-pieces of different powers are supplied. Those of English microscopes are distinguished by letters A, B, etc., while those of foreign makers are distinguished by numbers 1, 2, 3, and so on; the A of the former and the 1 of the latter having the lowest magnifying power. The *shorter* the eye-piece, the *higher* is its power. By many English writers a *short* is termed a "*deep*" eye-piece; the reader has therefore to remember that when "*deep eye-piecing*" is recommended by some writers, the employment of an ocular of *high magnifying power* is indicated. As the confusion is perplexing and altogether needless, the term "*deep*" ought to be abandoned.

Seeing that the eye-piece merely magnifies an image produced by the objective, any fault in the image produced by the latter is exaggerated. On this account, only very perfect lenses can bear a shallow eye-piece, and therefore its employment is of much service in testing the quality of

an objective. Generally speaking, it is well to keep to eye-pieces of medium power, such as the B or C of English makers and the No. 3 of Hartnack.

41. **Distance of the Ocular from the Objective.**—The magnifying power of the compound microscope may be varied by altering the distance between the objective and the ocular. Some microscopes are so constructed that this is impossible. Nevertheless it is *extremely convenient* to have the tube between the lens and the ocular made like that of a telescope (Fig. 1). One not unimportant advantage is, that such a microscope can be packed in a comparatively small box. The great advantage, however, is, that by varying the length of the tube very fine gradations of power can be obtained with the same lens and ocular. In measuring an object with an eye-piece micrometer it will afterwards be seen how important this is. The tube when fully elongated should not, however, reach more than ten inches above the stage, for it is often important in drawing and in measuring the magnifying power of the microscope to have the surface of the stage in the focus of the eye that is not looking through the microscope.

42. **The Bull's Eye Condenser.**—Opaque objects are seen by *reflected light*. The light is concentrated upon them with an ordinary condenser consisting of a plano-convex lens (*c*, Fig. 1). This may be fixed to a separate support, but in an ordinary microscope there is no place for it so convenient as that shown in Fig. 1. Considerable experience is required in the use of this apparently simple apparatus. It is important to bear in mind that the condenser should always, if possible, be placed at *right angles* to the direction of the light, and that, with a covered object, the light should fall on the cover-glass as directly as possible, for if it be very oblique it is to a large extent lost by reflection from the cover.

43. **The Mirror.**—Transparent objects are illuminated by *transmitted light* reflected from a mirror (N, Fig. 1). The mirror is usually slightly concave, and its distance from the object should be capable of alteration by a sliding or jointed movement, so that when working with a low

power the mirror may be placed at such a distance from the object as is necessary to illuminate the entire field, while with a high power the rays should be brought to a focus in or near to the object. The light is exactly focalised on the object, when a window bar, that may be brought into the field, can be seen sharp and distinct, or when the shape of a lamp flame can be distinctly seen. With artificial light it is often inconvenient to have the flame thus exactly focalised on the object, and therefore the lamp should be drawn a little nearer to the mirror to avoid this. The mirror should in general be so placed that the cone of rays is reflected from it at a right angle. One generally arranges the mirror so as to send the light *directly* through the object, but sometimes for the *resolution* of fine structure it is important to throw the light into the object *obliquely*. By such means one side of the object is thrown into shadow. In the more expensive microscopes the mirror has two surfaces, one concave, the other flat. The flat surface is used when the light is to be focalised on the object with an achromatic condenser (§ 52).

44. **The Diaphragm** usually consists of a blackened brass disc (M, Fig. 1) placed below the stage. It has apertures of various sizes, which can be successively brought under the aperture in the stage (L, Fig. 1). It is very important to attend carefully to the use of the diaphragm, and so regulate the amount of light transmitted through the object; for if a glare of light be allowed to pass through the object, the finer shadows are lost, and definition is thus rendered indistinct. With a high power a small aperture is employed. The diaphragm is, for convenience, usually placed about half-an-inch below the object. For lenses of very short focus, however, $\frac{1}{10}$ inch and upwards, it is very important to have the diaphragm close to the slide. In Hartnack's more expensive microscopes this is effected by a movable tube having one end partially closed by a diaphragm. Every objective has its appropriate diaphragm, the aperture being smallest for the lenses of shortest focus. The advantage of this arrangement is that the diaphragm can be pushed close up to the slide as it lies on the stage,

or withdrawn to any desirable distance. Nevertheless, it entails loss of time to shift the tubes, and *for ordinary work* nothing is so convenient as the circular plate. Its margin should be notched like a mill-headed screw, and there should always be a spring catch for arresting the rotation of the plate when any one of its apertures comes exactly under that of the stage.

45. **Employment of Daylight.**—The best light for microscopical work is that reflected from a white cloud. The light from the blue sky is not so good, and the light from gray clouds is about the worst. The window for microscopical work should, if possible, face that part of the sky opposite the sun at the time the microscope is used. On this account a north light is that which is most useful. If the microscopist is compelled to work at a window into which the sun shines, he should cover it with a white calico blind, for direct sunlight must never be employed. Good daylight is almost always to be preferred to artificial light; it often happens, however, that the latter must be employed. The mode of obtaining it will be considered in § 51.

It is of great importance to protect the eye from extraneous light. For this purpose a black paper or cardboard shade should be placed in front of the eye, so that as little light as possible may reach it, save through the microscope. With such a simple precaution one can work much longer without fatigue, and the sharpness of the microscopic image is much improved.

THE MECHANICAL PARTS OF THE COMPOUND MICROSCOPE.

46. **The Supports.**—The *pedestal* of the microscope may be of a tripod or of a horse-shoe form, it matters little, provided that perfect steadiness be obtained.

The *stage* (L, Fig. 1) should be a little broader than the length of a slide (3 inches), and it is very important that it be not too high. It ought to allow the forearm to rest on the table while the thumb and forefinger are moving the slide to and fro upon the stage. It is singular that English

opticians should have, for so long a time, sacrificed this primary consideration to the far less important one of occasionally fitting a large achromatic condenser below the stage. When the pillar of the microscope is bent, the glass slip must be prevented from sliding down the stage. Nothing does this so simply and so efficiently as a pair of clips made of *brass*, below which the ends of the slip are placed. A movable brass *ledge* sometimes is placed across the stage to serve instead of these clips; but a simpler contrivance for wasting one's time could scarcely have been devised. Movable stages are lauded by some; those with screws are scarcely worthy of serious attention, but the stage moved by a lever with a ball and socket joint, devised by R. and J. Beck, is —if a movable stage be desired—a good arrangement. At the same time, however, it is, I believe, much better to learn to do without any movable stage unless for very exceptional work under very high powers; in that case it is useful to those whose fingers have not acquired the necessary amount of dexterity to move the glass slip carefully. *Nothing is so convenient* as the motion communicated directly to the glass slip by the fingers. If the stage be kept smooth and clean—and a polished glass surface to the stage is on this account the best—the slide can be readily moved in any direction, and when working with very high powers it is only necessary to put the slip under the clips to keep it from moving too easily. With a little practice it is remarkable what a delicate movement may be given by the fingers.

The *pillar* of the microscope should be flexible, in order that the head may be kept in the erect posture, a condition that greatly diminishes the fatigue of a day's work. When working with fluids, the stage has of course to be made level. It is important to see that the hinge of the pillar be thoroughly well made, otherwise the stage will be unsteady.

The *telescope tube* holding the ocular and the objective has been already alluded to (§ 41).

A *nose-piece* (Fig. 32) is an excellent device for saving time in changing from one objective to another. In this simple contrivance, devised by Mr. Brooke, the low and high lenses are screwed to a framework which can be

rotated round a vertical axis, so as to bring the one or the other lens under the tube. In the original form of this apparatus both lenses remained vertical. Powell and Lealand, however, improved it by causing the lens not in use to project free from the stage. The form shown in Fig. 32 is a still further improvement made by Swift, in which the objective is readily centred accurately under the tube. The cap over the lens not in use should always fit closely to prevent the entrance of dust. The nose-piece may be made to carry *three* or even four lenses.

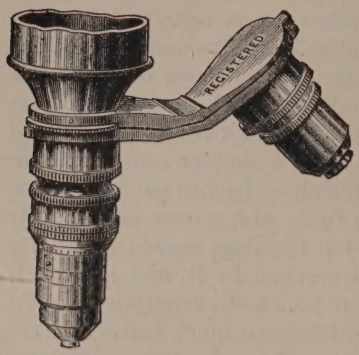

FIG. 32.—Nose-piece, made by Swift.

47. **The Adjustments.**—These are two in number, the *coarse* and the *fine*. The former moves the tube with the lenses rapidly, the latter moves it slowly up or down, so that the objective may be quickly brought to its focus with the former, and then very accurately by the latter. The fine adjustment is almost always a fine screw (H, Fig. 1); the fingers are constantly kept upon it during the examination of an object, in order to slightly change the focus and examine every stratum of it thoroughly. If, therefore, the screw be not sufficiently *low in position* to allow the arm to *rest on the table*, the fatigue becomes very inconvenient. The arrangement in Fig. 1 serves the purpose fairly well.

In such a microscope as that shown in Fig. 1 the *coarse* adjustment is effected by seizing the lower milled head on the tube (D), and giving it a rapid spiral motion. The only disadvantage of this is, that the tube is sometimes apt to jerk, and so to bring the lens suddenly down on the object. Moreover, unless the tube be kept quite clean, verdigris accumulates and it becomes stiff. Still, the pre-

caution of cleaning it with a cloth when one begins to work is easily attended to. And the difficulty can also be overcome by having the split tube that clasps the sliding body of the microscope lined with leather or cloth. If a nose-piece is not employed, this form of coarse adjustment is the best, because the tube can be readily pulled out of its split sheath when the lens is to be changed: for *nothing can be more inconvenient than attempting to screw or unscrew a lens with the tube fixed over the stage;* the hand being placed in an unusual position, the lens is apt to fall, and certainly much time is lost in getting the thread of the screw to bite when the hand is thus placed. *When a nose-piece is employed*, however, a coarse adjustment, consisting of a rack and pinion finely made, is the best arrangement, for then all jerking is avoided, and the spiral motion of the tube, which is disadvantageous with a nose-piece, is unnecessary.

FORMS OF MICROSCOPES.

48. **Monocular Compound Microscopes.**—Excellent microscopes are made in this country by Ross, R. and J. Beck, Powell and Lealand, Swift, Crouch, Collins, Baker, Browning, and others: in Paris by Hartnack, and Vérick: in Jena by Zeiss. Their forms are well-nigh endless, and cannot be considered here.* The main points required in any compound microscope are (1), good lenses; (2), perfect steadiness; (3), power of easy adjustment; (4), portability; (5), moderate cost. These qualities are wonderfully well united in the form of microscope (Fig. 1) which is very similar to that originally made at the request of the late Professor Bennett, by Oberhäuser, and now by his successor Hartnack, and also by Vérick of Paris. English opticians have been too much given to the manufacture of machines of imposing appearance. Now, however, they are wisely endeavouring to rival the cheap continental microscopes, and it is by no means improbable that ere long they will surpass continental makers in this direc-

* The reader is referred to Dr. Carpenter's excellent work on "The Microscope," 5th ed. 1875, for full information on this subject.

tion. What is wanted for ordinary microscopic work undoubtedly is an instrument resembling that shown in Fig. 1; it matters not who makes it, provided it be well made and at moderate cost. In the Edinburgh school, Hartnack's microscope has always been recommended hitherto, because it has met the requirements of the student better than other forms of the instrument. Quite recently, however, Mr. Swift of London sent for my inspection a student's microscope somewhat resembling Hartnack's. I was struck by the excellence of many of its parts. The objectives are $\frac{1}{6}$ inch and 1 inch, the former is of low angle, and the lens sent to me as a sample was certainly a better lens than the majority of Hartnack's No. 7. Its power was considerably higher. Swift's lens, however, wants a thin brass ring round its margin to give it more protection. His medium eye-piece (C), equal to Hartnack's No. 3, is excellent, but the shallow eye-piece (D), though better than Hartnack's No. 4, is of little use. With the $\frac{1}{6}$-inch objective and the C eye-piece it is possible by simply altering the length of the telescope tube to get a power ranging from 250 to 450 diam., a great convenience, and in this respect surpassing Hartnack. The pedestal, the hinge, the pillar, and the support of the body of the microscope, are all *steadier* than in Hartnack's III.A microscope* (Fig. 1). The fine and coarse adjustments are *certainly better* made. But the instrument is not yet perfect. The diaphragm is let into the stage from above, so that the disc rotates just under the slide; a faulty arrangement, with which it would be impossible to keep the stage clean. Acids getting between the diaphragm and the stage would spoil everything. The object of the arrangement is to have a rotating diaphragm with its apertures close to the slide, but until a

* Hartnack's No. III.A microscope, with objectives 3 (1 inch), and 7 (not quite $\frac{1}{8}$ inch), and oculars 3 and 4, may be obtained from Bryson, 60 Princes Street, Edinburgh, for £7 : 10s.; also from Tisley and Spiller, 172 Brompton Road, London, S.W.; and Baker, 244 High Holborn, London, W.C. Mr. Swift's (43 University Street, Tottenham Court Road, London) microscope above alluded to, with $\frac{1}{6}$-in. and 1-in. objectives, and C and D ocular, similar to Hartnack's, costs £6 : 12s.

more convenient stage and diaphragm are added, Hartnack's microscope will still be preferred. What is wanted is a stage of blackened glass. The clips upon Mr. Swift's microscope are faulty, and should be improved, and the condenser would be more convenient if fixed to the body of the instrument, as in Hartnack's microscope, and not placed on a separate stand, which always gets in one's way when working. The microscope, however, costs less than Hartnack's, and surely it will not be difficult to remedy its slight defects. When that is done, it will be a better microscope than Hartnack's. A nose-piece, and in that case a rack-and-pinion coarse adjustment, would render it a very perfect microscope for all ordinary work.

If a more expensive microscope stand be desirable, one similar to Hartnack's No. VII. stand should be procured. It is perfectly steady. The stage is covered with blackened glass. It has an easily worked rack-and-pinion coarse adjustment. The stage is not too high, and it—together with the tube and lenses—can be turned round a vertical axis, for the variation of the illumination of the object. Any English optician could make such a stand; meanwhile Hartnack's may be safely recommended.

49. **Binocular Microscope.**—If the rays transmitted from an object through each lateral half of the objective be focalised on the corresponding retina, so that two slightly dissimilar pictures are produced, the effect is stereoscopic; the object stands out *en bas relief*. Various methods have been invented for effecting this, most of which imply a permanent division of the tube of the microscope into two. Hartnack's arrangement is the best, for it is entirely confined to a special double-tubed eye-piece, which may at any time be placed in the ordinary monocular microscope. In this eye-piece there are four rectangular prisms (Fig. 33), in which the rays undergo total reflection. In two of these (A B) the rays from the objective are divided into two lateral bundles, that are again reflected by the prisms C and D, and focalised by the ordinary lenses of two eye-pieces at E and F. Such an arrangement may be satisfactorily employed with low powers, but with very high powers the loss of light entailed by the rays encountering so many glass surfaces, even at right angles, is a serious drawback, and definition is apt to be impaired. Therefore, although it is less fatiguing for the eyes to use the binocular, the monocular is still the microscope for ordinary work.

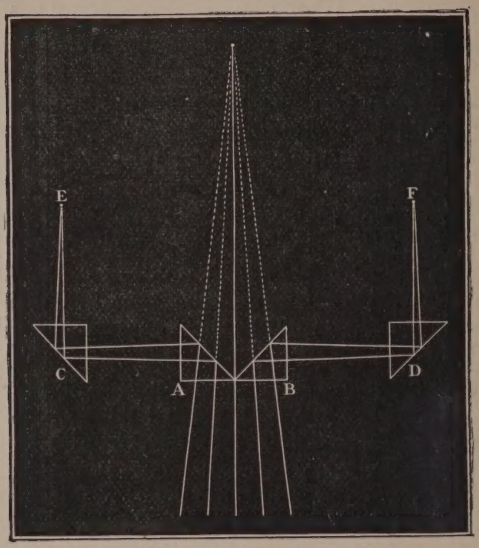

FIG. 33.—Course of the rays through Hartnack's binocular eye-piece. (*Ranvier.*)

50. **Dissecting Microscopes.**—The simple dissecting microscope devised by Darwin (Fig. 34) answers very well for ordinary purposes. Many, however, will prefer the binocular dissecting microscope made by R. and J. Beck, or Lawson's binocular dissecting microscope made by Collins.

A Brücke's lens (Fig. 35) is a very convenient form of simple microscope for assisting in dissecting and preparing microscopical specimens, and in making fine drawings. It consists of two plano-convex lenses, with their convex surfaces facing each other, and a concave lens above all. This arrangement increases the working distance between the microscope and the object. It has been adopted by R. and J. Beck in their binocular dissecting microscope.

ACCESSARY APPARATUS FOR ILLUMINATION.

51. **Lamp.**—If good daylight cannot be obtained, a lamp is necessary. Artificial light is far more fatiguing to the eyes than daylight, on account of the predominance of yellow rays. These may, however, be removed by trans-

mitting the light through pale blue glass. A plate of it may be placed on the stage of the microscope, or a blue chimney may be put on the lamp. Although the light is softened thereby, it is, however, at the expense of illumi-

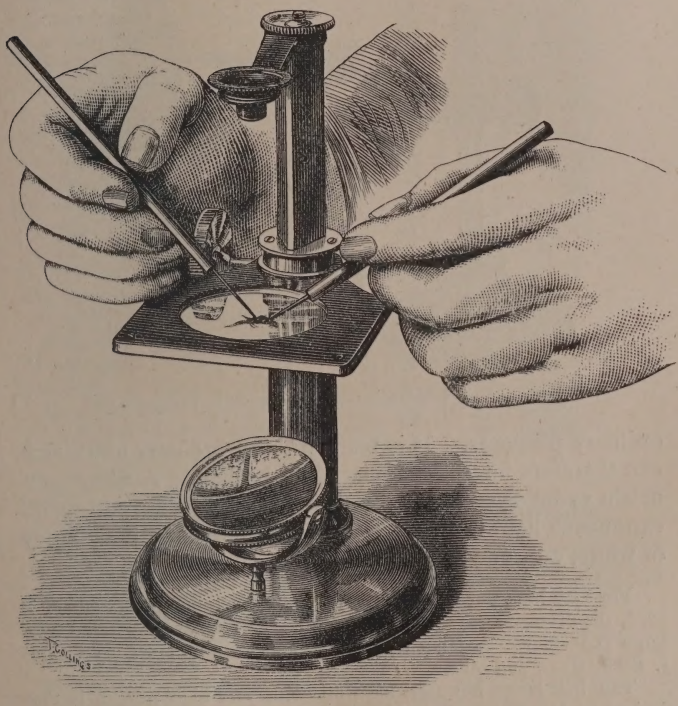

FIG. 34.—Darwin's dissecting microscope.

nating power, and therefore sometimes, with very high powers, the yellow flame has to be employed.

An Argand gas-burner may be used, and answers remarkably well if covered with a blue chimney. The light from a colza oil or a belmontine lamp is, however, softer. An ordinary colza oil reading lamp does extremely well; but if a special lamp has to be procured, a lamp for burn-

ing paraffin, or better still, the purified form of it named belmontine, is to be preferred. The simple form of lamp, devised by How of London (Fig. 36) serves very well for

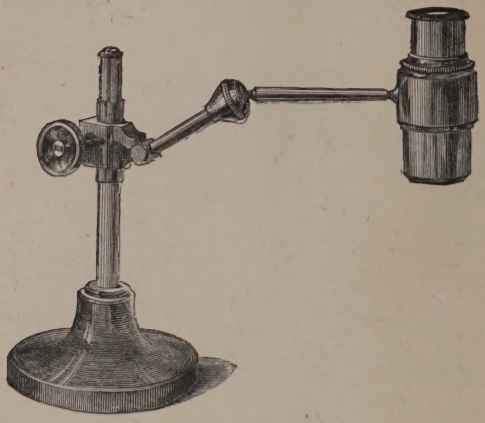

FIG. 35.—Brücke's simple microscope, made by Hartnack.

ordinary purposes. It is made with an ordinary bottle that can therefore be easily replaced, if broken. A white porcelain cylinder serves as a reflector, and also for cutting off extraneous light from the eye. It should have a chimney of white, and one of pale blue glass.

For very high powers, especially when an achromatic condenser is used, the diverging rays from the lamp must be rendered parallel by a large condenser. A lamp with this arrangement is made by R. and J. Beck, Ross, and others.

The best lamp, however, for work with very high powers is that devised by Mr. Dallinger, and made by Mr. G. S. Wood of Liverpool. (See *Monthly Microscopical Journal*, vol. xv. p. 165.) The lamp is expensive, but very perfect.

52. **Achromatic Condenser.** — This apparatus is of much service in illuminating objects when very high powers are employed. It may be simply made by fixing a half or quarter inch objective in a tube under the stage.

The flat surface of the mirror is used to reflect the light through the object, which must be arranged so that the light is focalised on the object. Special forms of this instrument, with diaphragm, having apertures of various sizes and shapes for giving different illuminating

effects, are admirably made by English opticians. As they are designed for their own microscopes, however, a special small condenser would have to be made for such a microscope as Hartnack's. (I cannot recommend Hartnack's achromatic condenser.) Mr. Dallinger's article "On a New Arrangement for Illuminating and Centering with High Powers," the reference to which is given in § 51, is well worthy of careful perusal in this connection. Mr. Dallinger and Dr. Drysdale have, by their remarkable researches on bacteria, shown how very thoroughly they have studied the points to be attended to for the proper illumination of microscopic objects under high powers.

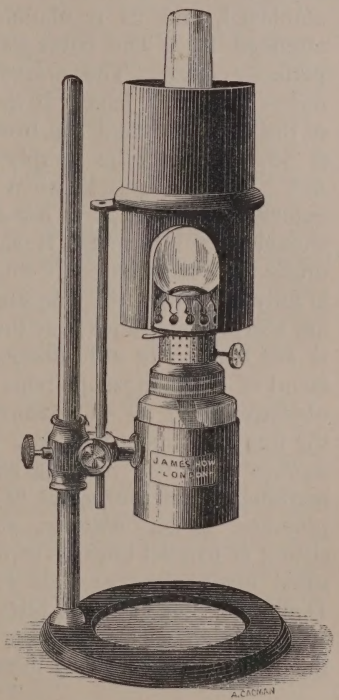

FIG. 36.—How's lamp for the microscope.

53. **Polarising Apparatus.**—This commonly consists of two Nicol's prisms. One, termed the *polariser*, is placed below the stage; the other, termed the *analyser*, is placed either immediately above the objective, or above the eye-piece. Hartnack's polariscope is perhaps the best. The analyser is placed in a special eye-piece, with a graduated circle and indicator to show the number of degrees through which the prism is rotated. The ray, polarised by the lower prism, can pass through the upper prism, when the principal sections of the two are parallel; but when they are placed at right angles, the polarised ray is arrested in the upper prism, and all is darkness.

If an object be placed on the stage and the Nicols be crossed, it is *invisible* if it refract light *singly*, but it appears *luminous* if it refract light *doubly;* the reason being that the object so twists the polarised ray that it can pass through the upper prism. Polarised light is used in the examination of striped muscle and other tissues, and various crystals.

THE METHOD OF TESTING THE MICROSCOPE.

54. **Test Objects.**—Before purchasing a microscope

it should invariably be tested by a practised observer. Clear daylight—from a white cloud if possible—should always be employed, and its regulation by the diaphragm carefully attended to. The cover-glass should be extra thin and perfectly clean. The *salivary corpuscle* is a good test-object for $\frac{1}{6}$-inch lens. If the margin of the nucleus, and of the corpuscle, and the Brownian motion of the granules (§ 86, *b*), cannot be sharply seen, the *definition is imperfect.* If the corpuscle, when exactly in focus, shows *any* colour, *the microscope is not achromatic. A drop of blood* should also be used. It should form a very thin layer under the cover-glass. If, when the tube of the microscope is fully elongated, the corpuscles in the centre of the field are sharp, while those near the periphery are misty, *the field is not flat.* The adjustments must work smoothly. The stand of the microscope must be perfectly steady, and the objective *must on no account move from side to side* when the fine adjustment is used. *A microscope should be rejected without hesitation for any one of these faults.* The fine markings upon the scales of Lepisma saccharina, Podura plumbea, various diatoms, and Nobert's test-object consisting of parallel lines of various degrees of approximation ruled upon glass, are all recommended as test-objects. Doubtless, they are good tests of defining power, but they are well-nigh useless as tests of achromatism, because of the dispersion which they themselves occasion.

THE METHOD OF WORKING WITH THE MICROSCOPE.

55. **Preparation of the Microscope.**—*a.* Select the objective and ocular required, and place them on the microscope. In the present case the $\frac{1}{6}$-inch lens (No. 7 of Hartnack) and an eye-piece of medium power (No. 3 of Hartnack) will be needed. See that their outer surfaces are clean, if not, rub them gently with soft wash-leather. If they are difficult to clean use dilute ammonia.

b. If the objective be the $\frac{1}{6}$-inch (No. 7 of Hartnack), place it *half-an-inch* above the level of the stage; if it be the 1-inch (No. 3 of Hartnack), place it *two inches* above the stage. In these positions they are of course *outside the focus.*

c. Arrange the mirror and illuminate the field of vision; use a small aperture of the diaphragm in the case of a high power. If any specks be visible on looking through the microscope, they are upon either the upper or lower glass of the eyepiece (probably on the upper surface of the latter), and must be removed. Muscæ volitantes must not be mistaken for such specks. The *latter* move as the eye changes its position. Dirt upon the *objective* is indicated by *no definite forms* on looking through the microscope, but *by a dimness of the object* which may be under examination.

d. Clean one or more cover-glasses. If the glass be new, use dilute alcohol. The cover-glass ought *never to be laid flat upon the table*, but should always be tilted on edge against some suitable support until required; otherwise, the surface to be applied to the object will be contaminated with various particles.

56. **Preparation of a Simple Object.**—Place a *small* drop of milk upon the centre of a slide. Hold the cover-glass by the *margin*, between the thumb and forefinger; rest the free margin nearest the finger-tips upon the slide, and allow the glass to fall, in this *oblique* manner, *gently* upon the object. If the cover-glass be *perfectly clean* the fluid will meet its surface evenly, if not, unmoistened islands will be left, and air thus entangled. By gently raising the margin of the cover-glass with a needle once or twice the air-bubbles may be got rid of. If too much fluid has been taken, remove it from the margin of the cover with bibulous paper.

In mounting specimens it is very important to allow the cover-glass to fall evenly, and without shifting its place, on the slide. This is best done with a pair of forceps (Fig. 37).

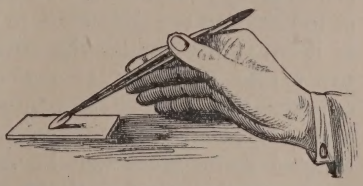

FIG. 37.—Application of the cover-glass.

57. **Description of the Object.**—It is very important that the student should be educated in the method of describing microscopic objects. To facilitate description, he should attend to the following:—

a. Shape. Are the milk corpuscles globules, or are they circular discs like coins? To determine this, touch the edge of the cover-glass with a needle, and cause the corpuscles to roll over and over, or effect this by inclining the stage. Farther, attend to this: when the focus is altered a globule comes into and goes out of focus *gradually*, while a flat corpuscle passes into and out of focus *suddenly*.

b. Size. Attend in this case to the *relative*, but omit the *absolute* size.

c. Border. Is it smooth and regular, or notched?

d. Upper and under surface. Elevate the objective, then slowly lower it, and carefully watch the upper surface as it comes into view. Is it smooth or rough? The character of the lower surface is unimportant in this case, but when a membrane such as the mesentery is under observation, it is important.

e. Colour. When exactly in focus, are the corpuscles coloured or colourless?

f. Transparency. Bodies may be *opaque*, *transparent*, or *semi-transparent*. The first transmits no light; through the second the outlines of subjacent objects are visible; the third transmits light, but the outlines of subjacent objects cannot be distinctly seen.

g. Contents. Does the corpuscle appear to be homogeneous, or can included particles be seen on carefully focussing through its whole depth?

h. Effects of re-agents. Add acetic acid and observe its effects on the corpuscles. For this purpose place a drop of the acid on the slide close to the edge of the cover-glass. If it fail to pass under the cover, dip a strip of bibulous paper into the milk at the margin of the cover-glass opposite the acid. The acid will then speedily penetrate and *irrigate* the corpuscles (see § 257). After it has affected them, those that lie near each other no longer remain distinct, but coalesce and form irregular masses, from which it is inferred that each milk globule has an envelope that is soluble in acetic acid. From the results of the chemical analysis of the milk, this envelope is believed to consist of casein, a substance soluble in an excess of acetic acid.

i. A knowledge of the *effects of re-agents* implies nothing less than an acquaintance with the majority of histological methods. In the course of the following demonstrations the student will more correctly appreciate the effects of re-agents by bearing in mind that they are applied to the tissues for the purpose of ascertaining—1. *Their chemical composition, e.g.* fat is blackened by osmic acid, and dissolved by ether; starch is coloured blue by iodine; earthy salts are dissolved by mineral and various other acids; albuminous tissues are rendered transparent, and may be partially dissolved by acetic acid. 2. *Their structural composition.* Many textures are so transparent and their outlines so faint, that great assistance in determining their structure is obtained by *staining* them with various substances:—*e.g.* a solution of nitrate of silver, by darkening the outlines of epithelial cells, brings into view what is otherwise invisible. Chloride of gold, by rendering nerve fibrils of a violet colour, often reveals them where they cannot otherwise be seen; carmine, magenta, and logwood, by staining nucleated protoplasts, are of the greatest service in showing their boundary lines. Also such substances as acetic acid, glycerine, and oil of cloves, by *rendering transparent* some parts of the tissues, permit of others being seen. 3. Some re-agents are applied for the purpose of studying their *effects on vital phenomena.* With this view heat, electricity, and various chemicals, are applied to corpuscles that exhibit movement—*e.g.* white blood corpuscles, cilia, etc.

58. **Delineation of the Object.**—It is very important that the student should embrace every opportunity of making drawings of microscopical objects, for drawing necessitates a thorough inspection of an object, and it impresses its features deeply upon the memory. The smooth but not too highly glazed "antique note paper" made by Pirie and Son, Aberdeen, is an excellent paper for drawing. The pencils used are H.B., 4 H., and 6 H. The shading should not be produced by lines, as in ordinary drawing and wood-engraving, but it should be a continuous shade produced by a rapid to and fro move-

ment of the pencil. This continuous smooth shading, so necessary for giving a fine effect, may be readily produced by rubbing the pencil drawing with an artist's "stump," consisting of a firm cone of soft paper on the end of a stick. The student should draw the object just as he sees it, without altering its apparent size. In making drawings of complicated objects, for the purpose of publication, it is often necessary to reduce the size, and to fill in the details of one drawing from many specimens, but with this the beginner has nothing to do. *The only practice advisable in his case is to draw things just as he sees them.* It is important to practise even the drawing of *outlines* without the aid of a camera. The details of objects must indeed always be filled in without such aid, nevertheless it is often difficult to get the exact size and the general form without a camera. To all drawings a statement of the magnifying power should be added, *e.g.* milk corpuscles × 300; and in the case of things very difficult to see it is well to state the objective and ocular employed, not only their numbers, but also the names of their makers.

a. Chevalier's camera lucida is an exceedingly good one (Fig. 38). The eye-piece of the microscope is removed, and the camera inserted in its place, and fixed in any position with a screw (*s*). The rays penetrate a prism (*p*), undergo total internal reflection, emerge, are collected by a special eye-piece, and penetrate a second prism (*p*), where they are reflected to the eye of the observer, to whom the object appears to lie upon the table. The outlines of the object may be traced with a pencil, as shown in the figure. It must be remembered, however, that the pencil *cannot possibly be seen through* the prism. The rays from the pencil have to pass its margin, and so reach the pupil. One-half of the pupil has therefore to be devoted to the rays from the prism, and the other to rays from the pencil and the paper. With Chevalier's camera the stage of the microscope is kept horizontal, so that it is perfectly serviceable in the case of fluids. The paper and the point of the pencil should be eight or ten inches from the eye. With a microscope having a tube ten inches long, the paper should be placed on a box on a level with the stage.

b. Single prism. Mr. Bryson of Edinburgh has recently fixed a small rectangular prism in a simple support, that can be readily clipped on the eye-piece of the microscope.

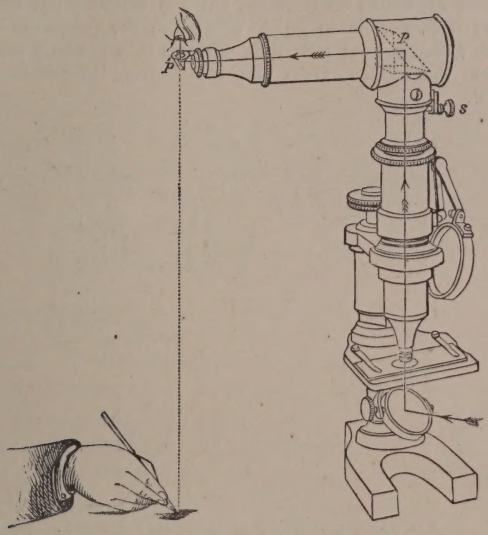

FIG. 38.—Chevalier's camera lucida, with Hartnack's microscope.

It acts like *p* in Fig. 38, gives as sharp an image as Chevalier's, and is far less expensive. The observer keeps his head in a *vertical* position, and makes the drawing on a suitable support placed vertically or slightly inclined ten inches in front of the eye. The observer should, therefore, sit at the left side of the microscope when it is directed towards the window, so that he may not obstruct the illumination of the mirror, nor get the direct light from the window into the eye that looks at the paper. The position of the hand in drawing is not quite so convenient as when it rests on the table, but really a camera is not very often employed, and the cheapness of the apparatus is a compensation.

c. Parallel plate reflector. A slip of thin glass placed above the ocular at an angle of 45° (*r*, Fig. 39), has long been recommended in

place of a prism. It may be fitted to a support that can be clasped to the eye-piece. A cover-glass answers the purpose. Dr. Beale, however, recommended that the glass should be of neutral tint, to diminish the glare from the paper, an advantage certainly. This is, doubtless, the cheapest apparatus for drawing, but it is certainly not so good as the prism; it cannot be so good, because in the prism the rays from the object undergo total reflection, whereas they are only partially reflected from the surface of the plate; moreover, the microscope must be inclined.

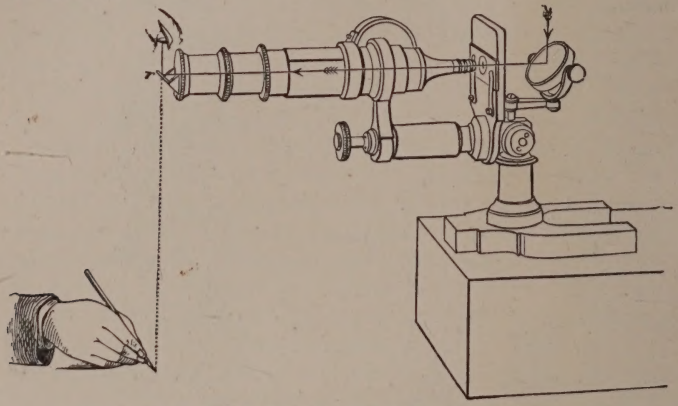

FIG. 39.—Drawing with parallel plate reflector, *r*.

Success in using *a*, *b*, or *c* will not, however, be attained unless the following be carefully attended to. The object must not be too brightly illuminated; a black pasteboard shade should be placed between the window and the eye to cut off extraneous light, and the paper on which the image appears to lie should be rather dimly lighted, otherwise the point of the pencil will not be clearly seen. *The beginner should always close one eye.*

d. Squared glass. A plate of glass with fine squared lines (Fig. 43) placed in the eye-piece is of much service for enabling one to draw objects of their exact size and form without a camera. The same piece of glass should be engraved, so as to serve as an eye-piece micrometer.

59. **Estimation of the Magnifying Power of the Microscope.**—A stage micrometer is necessary for the determination of the magnifying power of any combination

of objectives and oculars that may be placed on the microscope. Such a micrometer consists of a glass slide with a very fine scale engraved on it with the aid of a machine. In the English micrometer the divisions are $\frac{1}{1000}$ths and $\frac{1}{100}$ths of an inch. In the French micrometer they are $\frac{1}{100}$ths of a millimeter. Measurements are easiest with the latter.

Let the $\frac{1}{6}$-in. objective and the ordinary ocular be on the microscope (the No. 7 objective and No. 3 ocular in the case of Hartnack's microscope), with the tube fully elongated, what is the magnifying power? Place a millimeter micrometer on the stage, and focalise five of its larger divisions, the space included is 0·05 millimeter. The magnified image requires in some way or other to be compared with an ordinary hand millimeter scale to determine how much the 0·05MM has been magnified. Every one should try to do this as follows :—Hold the points of a pair of compasses ten inches from the eye (on a level with the stage of Hartnack's microscope), *keep both eyes open*, look at the micrometer lines with one, and at the points of the compasses with the other eye, open or close the points until they include exactly the five divisions of the micrometer, then apply the compasses to a millimeter scale. Suppose they cover exactly 15 millimeters, the magnifying power is ascertained by dividing the apparent size (15MM) by the real size (0·05MM).

Apparent Size. Real Size. Magnifying Power.

$$15\text{MM} \div 0{\cdot}05\text{MM} = 300 \text{ diameters.}$$

If an English micrometer be used, bring into focus a $\frac{1}{1000}$th inch division. Suppose it magnified to 0·3 inch, then;

$$0{\cdot}3 \text{ inch} \div 0{\cdot}001 \text{ inch} = 300 \text{ diameters.}$$

The use of compasses in this process is not essential; the millimeter, or inch scale itself, may be held at the side of the microscope on a level with the stage. The process, however, is much easier with compasses, because their points are more readily seen, and, unlike the lines of the scale, they are not apt to produce confusion.

By the above method the magnifying power can be ascertained with perfect accuracy; there is, however, an easier way, which may be had recourse to by those who possess a simple prism or a parallel plate reflector (§ 58, *b* and *c*). (Chevalier's camera is not here admissible, as it involves an increase of magnifying power by lengthening the tube.) If a scale be held *ten inches* in front of the prism, or the same distance below the parallel plate, the extent to which the micrometer divisions are magnified may be readily ascertained.

Having measured the power with the tube elongated, shorten it and estimate the power again. It will be found that the *shorter* the tube, the *lower* is the power. In this manner the powers of all the possible combinations of objectives and oculars belonging to a microscope should be ascertained and tabulated as follows.*

	Ocular.		
	No. 3.	No. 4.	
Objective.	Diameters.	Diameters.	
No. 3	50	65	Tube elongated.
No. 7	300	450	

The maker of the microscope generally supplies a table such as the above; but it is essential that the student should know how it is constructed, in order that he may test its accuracy, and measure the power of any lens that he may add to his microscope.

60. **Estimation of the Size of a Microscopic Object.**—This is done most easily and most accurately with an eye-piece micrometer (Fig. 40). This consists of a piece of glass with a fine scale engraved on it. It is most convenient to have a special eye-piece containing a micrometer, but it is also possible to get a micrometer adapted to the ordinary eye-piece. The ocular micrometer may be

* This table applies to a Hartnack's microscope, but it serves to indicate how the powers of any form of microscope may be tabulated.

in the form of a circular piece of glass, that is dropped into the eye-piece when required, or the glass slip may, as recommended by Mr. Jackson, be mounted in a brass plate capable of being pushed into the eye-piece through a slit in its side. The former is preferable, but it will not necessarily follow that, if the micrometer be dropped into the eye-piece, its lines will be clearly seen. They require to be exactly in the focus of the upper lens of the eye-piece, and to bring this about, an optician may have to fit the micrometer into a tube at such a height, that when it is dropped into the ocular, and rests on the stop, the lines will appear sharp and distinct. The micrometer lines usually form a series like that represented in Fig. 40, and for the purpose of ordinary measurement this is doubtless all that is necessary. It is, however, of great advantage to have the micrometer plate divided into squares, each square being the breadth of ten of the finest divisions of the scale (Fig. 43). A micrometer so constructed* is indispensable for counting the blood-corpuscles (Fig. 43), and also serves as the square of glass for drawing, alluded to in § 58 *d*. In measuring the breadth of the human coloured blood corpuscles, use the $\frac{1}{6}$-in. objective, elongate the tube, and substitute the micrometer in the ocular. Bring a single coloured corpuscle into focus. It is seen through the micrometer lines (Fig. 40). Suppose its broad diameter covers four of the smallest divisions: *push in the tube; the corpuscle does not now cover so many spaces; hence the value of the divisions of the ocular micrometer varies with the magnifying power*, and it is necessary to determine their value for all the powers employed. Therefore substitute a stage micrometer for the blood. If an English one be used, focalise a $\frac{1}{1000}$ inch division. Elongate or push in the tube until it covers just *ten* divisions of the ocular micrometer, then evidently each division must, with this magni-

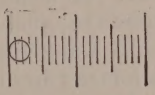

FIG. 40.—Ordinary form of eye-piece micrometer, with a human blood corpuscle, magnified 300 diam., seen through it.

* Such micrometers are made by Mr. Bryson, 60 Princes Street, Edinburgh.

fying power, be equivalent to $\frac{1}{10000}$ inch. This ascertained, substitute the blood for the stage micrometer. Suppose that the broad diameter of a coloured corpuscle covers three divisions of the ocular micrometer (Fig. 40), then $\frac{3}{10000} = \frac{1}{3333}$ inch. The corpuscles vary slightly in size; the average size being $\frac{1}{3200}$ inch.

61. It is very desirable that a uniform system of measurement should be adopted by histologists. We employ the metric system for fluid measures and weights, and it seems very desirable also to use it for measures of length. The expression of lengths in fractions of an inch is always clumsy, and apt to lead to confusion. The *micromillimeter* is the best histological unit; and it is very conveniently written by the Greek μ, and in speaking, shortened to *micro*. A micromillimeter is the 1000th of a millimeter (0·0000397 inch).

In using the millimeter instead of the English stage micrometer for the above observation, its divisions (0·01MM) will be seen through the lines of the ocular micrometer. Probably, with the magnifying power employed (300 diam.), it will be found that, with the tube somewhat shortened, *three* ocular micrometer divisions cover *one* division of the stage micrometer. If 3 oc. mic. divisions = 0·01MM; evidently 1 oc. mic. division must = 0·0033 *millimeter;* that is, 3·3 *micromillimeters*. With the tube of the microscope thus shortened, a red blood corpuscle would be found to cover very nearly 2·5 oc. mic. divisions; hence $3{\cdot}3 \times 2{\cdot}5 = 8{\cdot}25\ \mu$. The average size is $8\ \mu$. By remembering this figure for the blood corpuscle, it is easy to have an idea of the size of an object when expressed in micromillimeters.

PART III.

HISTOLOGICAL DEMONSTRATIONS.

62. **Granules and Brownian Movement.** (H.)—Rub a piece of gamboge in a drop of water on a slide until the fluid is *faintly* tinged; cover, and examine. The term *fine granule* is applied to a minute particle of any shape that has no light centre. The term *coarse granule* is applied to a particle of irregular shape having a light centre surrounded by a dark border. The difference between the optical characters of the former and those of the latter is entirely owing to size. Brownian motion is exhibited by all minute particles floating in a fluid. The motion is probably owing to thermal currents in the fluid; the solid particles merely indicating the directions of these currents.

63. **Hairs of Stamens of Tradescantia.***—*a.* With a pair of sharp-pointed forceps pull off a few hairs from a stamen of a flower (a white flower if possible) that is just opening. Place them in a drop of water on a slide; cover, and examine.

(L.) The hair is a chain of cells rounded at its apex, elongated towards its base. The youngest cell is at the apex.

(H.) Observe the envelopes, protoplasm, and nucleus of each cell. In the youngest cells the protoplasm fills the whole cavity. In the elongated cells there are irregular spaces (vacuoles) in the protoplasm, between which it stretches in the form of threads across the cavity of the cell, towards the nucleus. It also forms a more or less complete layer (primordial utricle) inside the envelope. The protoplasm is a colourless finely granular jelly. Ob-

* The Tradescantia Virginica is in flower from the middle of June until the middle of October.

serve its movements : in the threads, it streams in some towards, in others from, the nucleus. The whole mass of the protoplasm, clear substance as well as granules, moves.

b. Apply heat with the aid of a warm stage. The simple tin-plate described, § 259, Fig. 47, will answer the present purpose. A gentle elevation of the temperature hastens the protoplasmic movement, probably by accelerating chemical changes on which the movement in some way or other most likely depends. If the temperature be raised too high, however, the movement entirely ceases, owing to death of the protoplasm.

64. **Germinating Yeast.*** (H.)—*a.* Examine torulæ of various sizes. The vacuole seen amidst the protoplasm in the larger torulæ must not be mistaken for a nucleus.

b. Place a drop of magenta solution at the margin of the cover-glass. It stains the *buds* most rapidly. The protoplasm in many of the torulæ becomes stained, while the envelope *through which the staining agent must necessarily pass to reach the protoplast* remains comparatively colourless. A nucleus may be seen in some. The nucleus is generally the part of the cell which is most deeply stained by such agents as carmine, logwood, and magenta. Some torulæ never become stained. These are probably the oldest ones. Observe that the envelope is not equally thick in all the torulæ. In the youngest (smallest) bud no envelope can be seen. This arises either from the absence or from the excessive tenuity of the envelope. As the bud increases in size, the envelope becomes evident, and grows thicker with advancing age. The envelope is apparently

* If beer yeast (*Torula cerevisiæ*) cannot be obtained, procure German yeast, and sow it in sugar and water, or in the following modification of Pasteur's fluid :—

Magnesium sulphate	0·2 gramme.
Calcium phosphate	0·2 ,,
Potassium ,,	0·2 ,,
Ammonium tartrate	10 ,,
Cane sugar .	130 ,,
Water . .	800CC.

Keep the yeasty fluid gently warmed near the fire, or in a hot chamber for a few hours.

produced by the protoplasm. It is not a deposit from the surrounding fluid ; for it is known that the envelope of the vegetable cell consists of cellulose, and there is certainly no cellulose in Pasteur's fluid.

c. Rupture the torulæ. For this purpose make a pad of blotting paper, place it on the cover-glass, and beat it with the handle of a needle. Colourless ruptured envelopes will then be found scattered throughout the field, while the coloured protoplasm lies in masses here and there. Sometimes it is only partially outside the ruptured bag.

65. **Penicillium.*** (H.)—Examine in water. Observe that the hyphæ (stems and rootlets) consist of chains of elongated cells. Each aerial hypha (stem) produces at its summit four or five rows of small germ-cells (*conidia*) of a greenish colour. Even after staining with magenta no nucleus is rendered visible in any of the cells. (This fungus closely resembles the *Achorion Schönleinii* met with in the hair follicles in Favus.)

66. **Starch.** (H.)—*a.* Gently scrape the cut surface of a potato with a scalpel ; place the scraping on a slide ; add a drop of water ; diffuse the scraping in the water with the point of a needle ; remove all coarse particles ; apply the cover-glass ; and examine the starch corpuscles.

Observe their lines arranged concentrically around a bright refracting spot—the so-called "nucleus" of the corpuscle. This "nucleus" appears merely to be the first part of the concretion that is produced, the various layers being deposited concentrically around it. That the concretions are produced *within the protoplasm* of the cell, may be readily seen in a very thin section, with a power of about 800 diam.

b. Allow a drop of iodine solution (tincture of iodine 1 part, water 12 parts) to flow under cover-glass, and observe the blue coloration of the starch.

67. **Cotton Fibres.** (H.)—*a.* Place a few fibres of cotton wool on a slide in a drop of the above iodine solution ; cover, and examine. Notice the twist that is charac-

* The green dust of common mould should be blown upon the surface of modified Pasteur's fluid ten days or so beforehand. Fungi grow rapidly in this fluid.

teristic of these fibres. The fibres consist of cellulose. Unlike starch, it is not rendered blue by iodine, but acquires a brownish-yellow tinge like that of the iodine solution itself.

b. Allow a drop of dilute sulphuric acid (acid and water equal parts) to flow under the cover-glass. The fibres soon become swollen, and acquire a blue colour. *If the blue colour be slow in appearing, add a small drop of strong sulphuric acid to that already on the slide.* The reaction takes place equally well if the acid be added before the iodine.

68. **Linen Fibres.** (H.)—Examine in water. Unlike cotton fibres, they have no twist. They are for the most part solid, with here and there a crevice running along their interior.

69. **Disc-bearing Tissue.** (H.)—Make, with the aid of a scalpel, an excessively thin vertical section of an ordinary cedar pencil, or of a piece of common fir. Examine in water, and observe the disc-like depressions on the fibres.

70. **Bacteria.** (H.)—These may be readily developed in an infusion of some albuminoid matter—*e.g.*, muscle.

a. Mince some lean meat, and allow it to soak in cold water for an hour; squeeze it in the water with the hand; filter through calico, and then through paper. Place the filtrate in a hot chamber, or near the fire, to keep it at summer heat, for thirty-six hours or so. A pellicle forms on the fluid.

b. Place a particle of the pellicle with a small drop of the surrounding fluid on a slide; cover, and examine.

The pellicle consists of very minute, clear, motionless granules. These are young bacteria. They are imbedded in a clear jelly-like matter, that is, however, difficult to see unless a dye be used. In the fluid around the pellicle, the bacteria are in active motion. The minute granular bacteria dance about in directions that are *indefinite*, like the granules of gamboge already examined. The elongated bacteria exhibit, *as long as they are alive*, movements in a *definite* direction. They shoot across the field with great rapidity.

c. Add magenta. The fluid stains and kills them. The latter effect is probably due to the alcohol contained in the solution. When dead, they exhibit only Brownian movement. The direction of this movement is *indefinite.* The movement of the elongated bacteria in a definite direction results from contractility.

Examine the stained pellicle. The young granular bacteria are brightly tinged, while the jelly that surrounds them is very faintly so.

If the elongated bacteria, after staining with magenta, be carefully examined with a 1-25th inch lens, it may be seen that, as in the stained torula already examined, there is a brightly-stained matter in the centre, enclosed in a substance that remains colourless. The enclosed matter is doubtless protoplasm, and the enveloping substance is probably cellulose, because, like cellulose and unlike any soft albuminoid matter, it is unaffected by a solution of caustic potash. Dallinger and Drysdale (*Monthly Microscop. Journ.* vol. xiv. p. 105) have detected a minute *flagellum* or *cilium* at the end of the bacterium, which—acting like the tail of a fish—propels it through the fluid.

67, 68, 69, and 70 are examined because of the frequency of their occurrence in the urine and other fluids examined by the medical microscopist.

BLOOD.

71. **Blood of Newt.** (H.)—Stun the animal, dry the tail, snip a piece from its extremity, and then express a small drop of blood upon a slide. Place the cover-glass upon it immediately.

a. The coloured corpuscles.—*Shape* when seen in front face and in profile. *Size. Border. Colour. Transparency. Contents.* Observe that the corpuscles at first appear to be homogeneous, but that, after a time, a light oval spot appears in the centre of many; this is the position of the nucleus.

b. The white corpuscles.—There are several varieties of these. Some are very finely granular nucleated masses of protoplasm, as large, or even twice as large, as the coloured corpuscles. These are the *common* white corpuscles. Others are coarsely granular. These are not nearly so

numerous as the first. A third set consists of nucleated protoplasts, finely granular, and much smaller than the others. The nuclei can seldom be distinctly seen in the white corpuscles without the addition of re-agents.

c. The amœboid movements of the white corpuscles.—Select one of the large finely-granular corpuscles of irregular shape, and watch it carefully for some minutes. Usually, even at the ordinary temperature, it changes its shape with considerable rapidity, and it is instructive to draw the outline of the corpuscle at the end of every two minutes or so, in order to be fully convinced of the changes that occur. The direction of the motion is *indefinite;* a protrusion may take place at any part of the protoplasm, and be afterwards withdrawn, or the protoplasmic process may adhere to the slide or cover-glass, and the mass of the corpuscle be drawn after it, thus leading to a *locomotion* of the corpuscle. Sometimes neighbouring processes coalesce, and the readiness with which they do so is opposed to the idea that the protoplasm is enclosed in a membrane, as some are inclined to suppose.

d. Effect of acetic acid.—Place a small drop of dilute acetic acid at the margin of the cover-glass, and gently raise the cover with the point of a needle, to break the coagulum, and so allow the acid to penetrate. The coloured corpuscle suddenly becomes enlarged, but still retains its oval form. This probably results from softening of the envelope of the corpuscle, together with endosmose; the former permitting and the latter producing the enlargement. At the same time, the corpuscle loses colour, and the nucleus becomes evident. After a time it becomes very difficult to see the envelope of the corpuscle; the nucleus, however, remains perfectly distinct. It is usually somewhat corrugated. It sometimes happens that the nucleus becomes of a yellow colour, owing to the acid solution of the blood pigment tinging it, after the manner of such a dye as magenta. Until the acid is added, the nucleus is not permeated by the blood pigment. This lies entirely outside the nucleus, between it and the envelope of the corpuscle. The general mass of each *white corpuscle*

becomes transparent, and the nuclei very distinct. From three to five nuclei are usually revealed in the large corpuscles. The amœboid motion is arrested by the acid.

72. **Effect of Dilute Alcohol.**—Dilute alcohol (rectified spirit 1 part, water 2 parts) is a useful re-agent for the blood corpuscles (Ranvier). Under its influence the frog or newt's coloured corpuscle, and also its nucleus, become swollen, and a nucleolus is revealed. The nuclei of the colourless corpuscles become evident, and one or more very delicate clear blebs grow from the periphery of the white corpuscle, apparently consisting of some colloid substance, into which endosmose takes place rapidly.

73. **Effect of Water.**—Make a preparation of the same blood, and add water as described in § 71, *d*. The coloured corpuscles become more or less globular, owing to endosmose, and they lose colour owing to diffusion of their pigment into the surrounding fluid.

74. **Effect of Strong Syrup.**—Place a drop of the same blood on a slide, add a drop of strong syrup, and quickly mingle the two with the point of a needle, then apply the cover-glass. The coloured corpuscles shrivel, owing to exosmose. It is to be observed that some of the coloured corpuscles shrivel rapidly, while others undergo no change for a considerable time. Possibly the latter are older corpuscles and have thicker envelopes.

75. **Effect of Magenta.**—To a drop of the same blood add a drop of the special magenta fluid used for staining blood corpuscles (§ 324, *b*). Mix the two with the point of a needle, and then cover. The nuclei of coloured and colourless corpuscles become brilliantly stained. The magenta fluid here employed contains a quantity of glycerine sufficient to render its specific gravity similar to that of the blood, with a view to prevent the swelling of the coloured corpuscles. If the coloration take place slowly, however, a drop of the ordinary watery magenta fluid may be added to facilitate the penetration of the corpuscle by the dye.

76. **Effect of Tannic Acid.**—Dissolve 5 decigrammes tannic acid in 113CC hot water, and allow it to become cold. Place a drop of the same blood on a slide, and cover. Bring into focus the corpuscles near the right margin of the cover-glass, and place as quickly as possible

a drop of the tannic acid solution near this point. The coloured corpuscles often become globular, and after a time the hæmoglobin becomes slowly or suddenly extravasated from the corpuscle in the form of one or more buds adherent to the wall, or in the form of fine granules scattered irregularly around the wall. With 1-25th inch objective it may be seen, after the action of tannic acid, that the coloured corpuscle has a definite envelope, and this envelope may sometimes be found ruptured where the bud has formed. With such a power as this one may see the pigment rolling away from the envelope like a cloud. It is not always extravasated, but may collect around the nucleus in a stellate mass. This effect of tannic acid was discovered by Dr. Roberts of Manchester. A 2 per cent solution of boracic acid (Brücke) has a somewhat analogous action. For §§ 73, 74, and 75, frog's blood does perfectly, but for § 76 newt's blood is preferable.

77. **Blood of Bird** (H.)—*a.* Observe the pointed, oval, biconvex shape of the coloured corpuscles, and their much smaller size as compared with those of the amphibian. A colourless corpuscle may be seen here and there.

b. Add acetic acid. A nucleus is revealed in the coloured corpuscle.

78. **Blood of Fish** (H.)—This blood must be examined in a perfectly fresh condition. The corpuscles resemble those of the bird. The coloured corpuscles, however, are not quite so pointed.

79. **Human Blood** (H.)—Take a small drop of blood from the finger, cover it immediately, and find the focus as quickly as possible.

a. The coloured corpuscles.—Observe them running together to form rouleaux. Study an isolated corpuscle. *Shape;* when seen in front face and in profile. *Size. Border. Colour. Transparency.* Observe in a corpuscle —seen in front face—the dark spot in the centre surrounded by a light margin, and that when the focus is changed, the centre becomes light and the margin dark. This results from the biconcavity of the corpuscle.

b. The white corpuscles.—Few in number; found singly

or in groups of two or three here and there amidst the rouleaux. Their shape is irregular, and amœboid movement may possibly be seen. Generally, however, the blood requires to be raised to its normal temperature (38° C.) with the warm stage (Fig. 48 or 49) before the movements can be clearly seen. The size of the white corpuscles varies. Some are twice as large as the coloured corpuscles, others are about the same size.

c. Fibrin. — Very delicate colourless homogeneous threads of fibrin may possibly be found stretching across the spaces between the corpuscles.

d. Effect of acetic acid.—Add acetic acid at margin of cover-glass, and if necessary gently raise the cover with the point of a needle to break the coagulum. Observe that the coloured corpuscles become globular, and lose their colour, while the white corpuscles become very transparent and most of them exhibit three nuclei. Sometimes, however, though rarely, there is only a single nucleus, and sometimes there are as many as five.

80. **Crenation of the Corpuscles.**—Allow a drop of the same blood to remain exposed to the air for twenty seconds or so before applying the cover-glass in order to produce crenation of the coloured corpuscles. A corpuscle thus changed is covered with delicate spinous projections, like a thorn-apple. In most bloods only a few of the corpuscles become crenated, while in other specimens nearly all the corpuscles undergo this change. Possibly those corpuscles which are prone to crenate have thinner envelopes than the others, and on this account more readily permit of the slight exosmose to which the crenation is commonly ascribed. There is reason for suspecting, however, that crenation may be due to some other cause as yet unknown; for it is regularly produced in such an animal as a dog when poisoned with Calabar bean (Fraser), and in that case it seems unreasonable to ascribe it to any change in the specific gravity of the blood plasma or corpuscles.

81. **Effect of Magenta.**—Add to the same blood, *before applying the cover-glass*, a drop of the special magenta fluid for staining blood corpuscles (§ 324, *b*); mix

the two with the point of a needle, and cover. No nucleus is revealed in the coloured corpuscle, but in the white corpuscle three nuclei usually appear.

82. **Effect of Tannic Acid.**—Add to the same blood a drop of tannic acid solution (§ 76). Mingle the two with the point of a needle; cover, and examine immediately. The coloured corpuscles undergo a change similar to those noticed in the newt's blood. Frequently, however, a delicate envelope may be seen outside the bud of extravasated hæmoglobin.

83. **Structure of the Blood Corpuscles.**—In all animals the white blood corpuscles are nucleated masses of protoplasm without any apparent envelope. The coloured corpuscles are nucleated in fishes, amphibians, and birds, but not in mammals; and it is to be observed that the mammalian corpuscle comports itself towards re-agents *not like the nucleus, but like* the *perinuclear portion* of the nucleated coloured corpuscles. Notwithstanding the contrary opinion of Rollett (Stricker's *Histology*, i. p. 408), we maintain with Hensen (*lib. cit.*, p. 409) that the coloured corpuscles have an envelope. The membrane can be distinctly seen with a power of about 1000 diam. in the newt's corpuscle after the addition of magenta and tannic acid. In the mammalian corpuscles it is difficult to form a decided opinion from direct observation, but the behaviour of the mammalian corpuscle towards re-agents is so similar to that of the newt's corpuscle, that the existence of a membrane in the former may be safely inferred. The hæmoglobin lies between the nucleus and the envelope. All that is to us perfectly clear, but the remainder is doubtful. Rollett maintains that the pigment fills the interstices of a colourless sponge-like stroma. Hensen (quoted by Rollett) states that he has seen a system of extremely delicate filaments stretching from the nucleus of the newt's corpuscle outwards to the envelope. Whether or not this sponge-like or thread-like stroma is present it would be difficult to say, but its existence would assist us in comprehending how the non-nucleated human corpuscle so definitely and regularly maintains its biconcave shape.

83A. **Effect of Gases.**—The influence of a stream of carbonic acid upon the coloured corpuscles may be studied by Stricker's method. A drop of water is placed on the floor of the chamber in his hot stage (Fig. 48), and a cover-glass, on which a drop of newt's blood has been previously placed, is inverted over the chamber. A ring of oil is painted round the margin of the cover to prevent evaporation. The stage is then gently heated until the water in its moist chamber evaporates, and the corpuscles at the periphery of the drop become affected by the moisture. When the red corpuscles are just about to lose their colour, the further addition of heat is discontinued, and a stream of carbonic acid is driven through the chamber by the apparatus described in § 258 (Fig. 46). If the coloured corpuscles have been sufficiently acted on by water, the carbonic acid produces a cloudiness within them,—which, according to Schmidt and others, is due to the precipitation of paraglobulin. The precipitate disappears if a stream of oxygen or common air be substituted for the carbonic acid.

83B. **Effect of Electricity.**—Make a preparation of human blood on a slide with gold leaf electrodes (Fig. 51), and transmit shocks through it from a Du Bois Reymond's induction apparatus, in connection with a voltaic cell as powerful as a small Grove's element. The secondary should be pushed close up over the primary coil of the apparatus. The electricity causes the white corpuscles to withdraw their processes, and to become more or less globular. The coloured corpuscles become successively crenated, globular, and finally colourless, owing to a diffusion of their pigment into the surrounding stroma. A similar effect is produced by discharges from a Leyden jar. These effects, first shown by Rollett and Neumann, are not entirely explained, but in all probability they chiefly result from electrolysis; although induced is not nearly so powerful as voltaic electricity in giving rise to electrolytic effects.

83C. **Inclusion of Solid Particles by the Colourless Corpuscles.**—As in the case of an amœba, a white blood corpuscle can entangle and envelope solid particles. This is effected by protrusions of the protoplasm growing around the particles. To demonstrate this phenomenon, human or newt's blood may be employed; the latter is, however, to be preferred, on account of the larger size and greater number of the white corpuscles. Fine granules of carmine, vermilion, indigo, or aniline blue that has been precipitated by alcohol from a watery solution, may be employed. (Schultze, *Schultze's Archiv*, vol. i. p. 1.) These substances are reduced to a very fine state of division, mixed with aqueous humour, and introduced into the blood, which may be previously diluted with an equal bulk of aqueous humour. In the absence of aqueous humour, dilute albumin (white of egg 1 part, $\frac{3}{4}$ per cent salt solution 2 parts, strained through muslin) may be employed. The blood with the pigment is placed on a slide and covered. Evaporation is prevented by a ring of oil painted round the cover-glass, and the preparation is kept on the warm stage (Fig. 49), at 30° C. in the case of newt's blood, and at 38° C. if the blood be human.

Cohnheim proved the practical importance of feeding the white blood corpuscles with these pigments, by injecting for several days in succession 2 or 3CC of water with fine particles of aniline blue, into a subcutaneous lymph sac of the frog. The lymph corpuscles enveloped the blue granules and entered the circulation. After some days he induced inflammation of the cornea, and found among the pus-cells at the seat of irritation, some containing the blue granules, proving that they had emigrated from the blood-vessels into the corneal tissue.

83D. **Enumeration of the Blood Corpuscles.** — Malassez and Potain have devised a method for estimating the number of coloured corpuscles in a given quantity of blood (*Archives de Physiologie*, 1874, p. 32), a matter of much importance both in physiology and pathology.

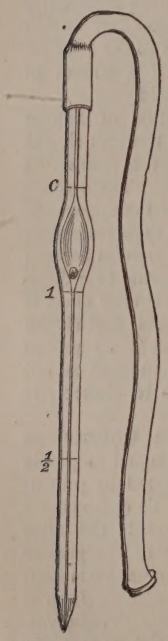

FIG. 41.—Pipette for diluting blood with artificial serum. (*After Malassez.*)

The blood is diluted with an artificial serum in order to prevent coagulation, and to facilitate the counting of the corpuscles. The artificial serum consists of 1 vol. watery solution of gum arabic, having a Sp. G. of 1020, 3 vols. watery solution of equal parts of sodium sulphate and sodium chloride, having a Sp. G. of 1020. The mixture of the blood with the above serum may be made in an ordinary CC measure, but if the quantity of blood be small, the pipette shown in Fig. 41 is essential. This is a glass pipette with a capillary tube, and a bulb containing a glass bead for mixing the blood and serum. An elastic tube, about a foot in length, is attached to one extremity. In estimating the number of coloured corpuscles in human blood, the finger is pricked, and a large drop of blood expressed, the elastic tube is placed in the mouth, the pointed end of the pipette in the drop of blood, and the fluid is sucked into the tube until it exactly reaches the line marked 1. The point of the pipette is then placed in the serum, and the aspiration continued until the fluid exactly reach the line *c*. During the entrance of the serum, the pipette is shaken, so that the glass bead may cause its thorough admixture with the blood. The instrument is so graduated that the cavity of the pipette below the line 1 contains exactly $\frac{1}{100}$ part of the cavity of the bulb from the line 1 to the line *c*. The mixture of blood and serum, therefore, contains one per cent of blood. In cases where the blood corpuscles are very numerous, it is advantageous to have only half a per cent of blood in the mixture. In such a case the aspiration of the blood into the pipette is arrested when the line marked $\frac{1}{2}$ is reached, and the serum is added as before. One must be careful to remember that after the mixture is made, the fluid in the long stem of the pipette is merely serum, and must be expelled as useless.

The diluted blood is now introduced into a capillary glass tube (Fig. 42) flattened and fixed to a glass slide. Near the upper surface of this

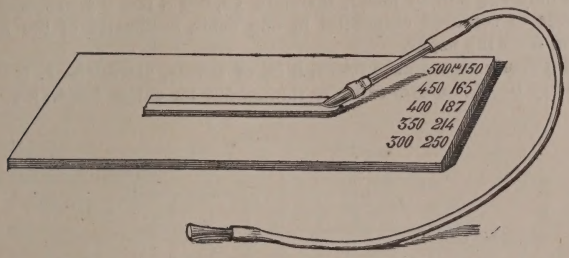

FIG. 42.—Capillary tube for enumeration of blood corpuscles. (*After Malassez.*)

tube there is an exceedingly fine elliptical cavity, the capacity of various lengths of which is carefully ascertained by the maker. The different lengths are tabulated in micromillimeters (§ 61) in the left column of numbers engraved on the slide (Fig. 42), while the capacities of these various lengths are expressed in fractions of a cubic millimeter in the right column; *e.g.* 500 μ contain $\frac{1}{150}$ of a cubic millimeter.

The capillary is filled with the diluted blood by placing a drop at the extremity, and allowing it to enter by capillary attraction. It may be necessary, however, to hasten its entrance by aspiration. As soon as the glass tube is filled, the remainder of the drop must be carefully removed by bibulous paper, otherwise the corpuscles will not come to rest within the tube.

Suppose we desire to count the corpuscles in 500 micromillimeters of the capillary, we proceed thus:—A millimeter stage micrometer is focalised by a power of about 100 diameters. A squared micrometer (Fig. 43) is placed in the eye-piece, and the tube of the microscope is shortened or lengthened until the whole breadth—viz. ten squares—of the ocular micrometer exactly include 500 micromillimeters of the stage micrometer. It avoids future trouble if a circular line be drawn on the tube of the microscope to indicate its position when the ten squares of the ocular micrometer have this value, with a given combination of lenses.

The capillary tube with the blood is now substituted for the stage micrometer, care being taken not to alter the length of the tube of the microscope. Fig. 43 represents the capillary seen through the squared micrometer. The coloured corpuscles within the whole of the 500 micromillimeters of the capillary have now to be counted. The squares in the eye-piece facilitate the process, and the number found in each square should be tabulated to avoid error.

Suppose that 334 coloured corpuscles are counted in 500 micromillimeters of the tube, this would, in a tube of the calibre of that under consideration, be the number in $\frac{1}{150}$ part of a cubic millimeter

of the diluted blood. Evidently then, $334 \times 150 = 50,100$, the number of corpuscles in one cubic millimeter. But as this diluted blood contains only $\frac{1}{100}$ part of blood, therefore $50,100 \times 100 = 5,010,000$, the number of coloured corpuscles in one cubic millimeter of the blood drawn from the finger.

To secure an accurate result, it is, of course, essential—1. that the capillary be of uniform capacity throughout its entire length; 2. that

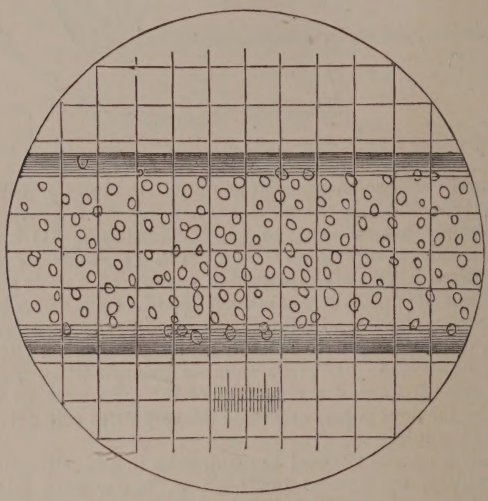

FIG. 43.—Capillary tube with diluted blood, seen through a squared ocular micrometer, × 100 diam. (*After Malassez*).

the blood be thoroughly mixed with the serum; 3. that the enumeration be accurately performed.

The average number of coloured corpuscles in a cubic millimeter of healthy human blood is 5,000,000. The number may be as high as 6,000,000, and in diseased conditions it may fall as low as 800,000 (Malassez, *lib. cit.*)

The method for counting the white corpuscles is essentially the same as the above; but, owing to their smaller number, the mixture of blood and serum should contain *two* instead of one per cent of blood. This is readily effected as follows:—Fill the pipette with blood to the line 1, then aspirate air until the blood enters the bulb. Aspirate the same quantity of blood a second time, and then mix the blood with serum as before. The average number of white corpuscles in a cubic millimeter of human blood is about 9000, that is about $\frac{1}{600}$ of the number of the coloured corpuscles.

84. **Blood Crystals** (H.)—*a. Hæmoglobin* may be readily prepared as follows:—Kill a rat or mouse by the inhalation of ether. Place a drop of its blood upon a slide, and add twice its volume of water. Mix the two with the point of a needle, and allow slight evaporation. Prismatic crystals, either isolated or in rosettes, will be found. These may be preserved by allowing the blood completely to dry before the application of the cover-glass, and then applying a drop of dammar solution and covering.

b. Hæmin crystals are most readily prepared in considerable quantity as follows:—Place some finely-powdered dried blood in a test tube; add about one-twentieth of chloride of sodium, and about ten times its bulk of glacial acetic acid. Heat, and keep it a temperature not higher than 50° C. for three or four minutes (the solution should not be boiled). Filter and evaporate slowly.

MUCUS.

85. (H.)—Mucous corpuscles may be obtained by gently scraping the interior of the trachea of an animal recently killed, or by hawking up some mucus from the back of the throat. They have usually a single globular nucleus; sometimes, however, there are two or three. They exhibit amœboid movement, especially if they be warmed to 38° C. on the hot stage (Fig. 49). Mucous corpuscles may also be obtained from the urine by allowing it to stand in a conical glass for six or seven hours until the corpuscles are deposited. If the greater part of the supernatant fluid be poured off, they can then be readily obtained with a pipette (Fig. 45). Those from the urine exhibit no amœboid movement, even if they be warmed; probably because of the paralysing influence of the water or other urinary constituent.

EPITHELIUM.

86. **Stratified Squamous Epithelium** (H.)—Move the tip of the tongue roughly over the gums and interior of

the lips to detach some epithelial scales; then place a large drop of saliva on a slide, skim off the air-bubbles with a needle; cover, and examine.

a. Squamous epithelial cells, single or cohering together in groups, will be found scattered throughout the field. Observe their shape, large size (30-50 μ), the nucleus usually single and oval.

The epithelium of the mouth is stratified. The cells under examination belong to the most superficial layers.

b. Salivary corpuscles will be found singly or in clusters. Size, rather larger than that of a mucous corpuscle. Observe the Brownian motion of the granules between the nucleus and the envelope. There is no amœboid movement.

c. Mucin.—Place a drop of magenta at the margin of the cover-glass, and elevate it slightly to allow the dye to penetrate the viscous fluid. The nuclei of the epithelium and salivary corpuscles become brilliantly stained, and very delicate films or fibrils of precipitated mucin will probably be found stretching here and there across the field. The mucin is precipitated by the alcohol in the magenta fluid, and as it is slightly stained by the dye it may be found without much difficulty. Acetic acid may be used to precipitate the mucin, but the films being colourless are, in that case, much more difficult to find.

87. V. S. skin of palmar surface of finger hardened first in chromic acid and spirit (§ 16). The skin may be imbedded in paraffin, as described in § 300, and held in the hand during section; or it may be imbedded in paraffin and cut in a microtome, as described in § 303; or, best of all, it may be frozen, and cut in the freezing microtome, as described in § 305. However obtained, the section must be extremely thin. If the slice be large and difficult to spread out on the slide, put in a tumbler or beaker full of water. Before it has had time to sink to the bottom thrust the slide into the water, and with the aid of a needle draw the section upon the centre of the slide, with the epidermic surface facing the broad margin of the slide. Dry the slide with a cloth or bibulous paper close up to the tissue.

Apply a drop of Farrants' solution or glycerine; cover, and examine.

(L.) The epidermis consisting of squamous cells roughly divided into two layers, the horny layer above and the rete mucosum below. The *cutis vera* with its papillæ. Select an extremely thin piece of the rete mucosum close to the cutis vera; place it exactly in the centre of the field, and fix the slide in that position with clips.

(H.) The epidermic cells are produced by fission in the lowest layer of the rete mucosum. Observe the small size of the cells there, as compared with the upper part of the rete, where they are *growing* but *not dividing*. The margins of all the cells are jagged. This can be clearly seen in the rete mucosum, and in the lower part of the horny layer. Towards the upper part of the horny layer the cells become scaly, and the nucleus often disappears. In the lowest layers the cells appear to be nucleated protoplasts. As they grow old they become horny, owing to the production of keratin, a substance into which the protoplasm is probably transformed.

The history of the epithelium lining the mouth is similar to that of the epidermis. As it is more difficult to see the jagged margins of the cells in the former, the latter is taken here. (*This preparation will be required again when we study skin.*)

87A. The evidence of the fission of the cells in the deepest layers of the epidermis can only be clearly seen with a power of about 800 diam. The nuclei of the cells are generally elongated, and mostly contain more than one nucleolus. Fission of the nucleus may sometimes be seen. It cannot be doubted that the perinuclear plasm also divides, but it is astonishingly difficult to see a cell in the act of division, probably because they are so closely packed together.

87B. The serrated margins of the cells are well seen in skin of human finger, treated by a method which we owe to Ranvier. Inject $\frac{1}{4}$ per cent osmic acid (§ 281) into the lower part of the epidermis by thrusting the nozzle of Wood's syringe (§ 344) obliquely into the cutis vera, and driving the fluid wherever it will go. Harden in rectified spirit, make *V. S.*, and preserve in glycerine. The lowest epidermic cells are slightly darkened, and a power of about 800 diam. shows that the so-called "prickles" are not the teeth of neighbouring cells "dove-tailed one with another," but that, as Dr. Martyn (*Monthly Microscop. Journ.*, xiv., p. 59) has shown—they are bridges between the cells

with intervening spaces. One may correctly imitate the arrangement by expanding the fingers of both hands and apposing their tips. Possibly the spaces constitute a system of lymph channels.

87c. The "prickle" cells may be isolated by teasing sections of the skin after prolonged maceration in $\frac{1}{6}$ per cent potassium bichromate, to which one or two drops of carbolic acid have been added to prevent putrefaction (§ 289). They may also be readily obtained (Lister) by placing one's foot in hot water for half-an-hour, and then scraping off the epidermis, and macerating it in the above bichromate solution.

87d. **Simple Squamous Epithelium.**—Omentum of full-grown cat or guinea-pig, previously silvered by the method described in § 328. Excise a small piece of the membrane with scissors, and extend it on a slide by the method described in § 87. Apply a drop of glycerine; cover, and examine.

(L.) The membrane is at some parts areolar, at others continuous. In the non-areolar portions, chains of fat cells may be recognised, and it will be just possible to see a fine network of dark lines, the silver lines that surround the epithelial cells. The omentum is a thin serous membrane with a simple layer of squamous epithelium on both surfaces. Between the epithelial coverings there is fibrous tissue, and at the trabeculæ there are, in addition, fat cells, bloodvessels, and nerves.

(H.) Examine the silver lines upon the trabeculæ. The silver blackens what appears to be an interstitial substance that cements the cells together. If, however, the tissue have been for more than three or four minutes in the silver solution, the nucleus, and, to a less extent, the perinuclear part of the scale, become darkened. Over the bands of tissue between the spaces in the areolar portion of the membrane, the silver lines are often imperfect. Profile views of the nuclei of the epithelial plates that clasp the fibrous bundles in this situation will probably be readily seen.

Seal up the preparation. All glycerine projecting beyond the margin of the cover-glass must be removed with a pipette (Fig. 45), and, if necessary, the slide must be washed with bibulous paper dipped in water. Paint a ring of gold size around the margin of the cover-glass, and

next day, when this has dried, cover it with white zinc cement (see § 358).

(This preparation will be required for fat cells and areolar tissue on a future day.)

88. **Ciliated Epithelium.**— *Ciliary Motion* may be readily studied in the gill of the mussel. The salt-water mussel is much to be preferred to the fresh-water one. In opening the shell, collect the salt water in a watch glass for future use. The gill consists of parallel bars, the free ends of which are at its free margin.

a. With scissors and forceps remove a small piece of the gill, including its free margin, place it on a slide, and apply a drop of sea water. As the gill in each half of the animal consists of a double membrane, separate the two portions with a pair of needles (a dissecting microscope (Fig. 34) is here of service), and then very gently pull asunder a few of the bars of one of the membranes; apply the cover-glass; place the preparation on the tin hot stage (Fig. 47), and examine.

(L.) Observe the bars with their free rounded ends covered with cilia moving "like a field of corn before the wind." Particles in the surrounding fluid are propelled in a definite direction, the same as that in which the cilia bend.

(H.) The cilium tapers from base to tip. (When motionless, and examined with a power of about 1000 diam., it is seen to be a filament, flat, perfectly transparent, and apparently homogeneous.) The cells on which the cilia are placed cannot be clearly recognised in this preparation, but the clear band at the margin of the cells, from which the cilia spring, may be distinctly seen. The movement of the cilium has a definite direction, consisting in its becoming alternately bent and straight. The propulsion of the surrounding fluid in the direction of the bend may be accounted for by supposing that the velocity with which the cilium bends is greater than that with which it regains its erect position.

b. Effect of heat.—The hot stage is already under the slide, apply heat as in the experiment with Tradescantia

(§ 63, *b*). A slight increase of temperature accelerates the ciliary motion, probably by hastening the chemical changes on which it depends. A too high temperature arrests the movement, doubtless by destroying the delicate molecular machinery of the contractile substance.

89. **Effect of Chloroform** (H.)—To study the effect of chloroform vapour on ciliary motion, first shown by Mr. Lister, take a slide with a glass cell (Fig. 62, *b*) or make a cell with a ring of putty, and place it on a slide. Place a small piece of the mussel's gill in a drop of sea water on a cover-glass. Put a *small* drop of chloroform in the cell; invert the cover-glass over it, so that the preparation will hang into the chamber. Find the focus quickly, and observe that the ciliary movement gradually comes to a standstill. The chloroform molecules evidently penetrate the contractile substance of the cilia, and inhibit or restrain those chemical or other changes in its molecular machinery that are essential for the contractile movement. If—when the movement is nearly at or has just come to a standstill—the cover-glass be removed, the cell washed out, and the preparation exposed for some time to the air, the chloroform molecules escape, and the movement is resumed. This experiment may be more easily performed with the aid of a gas-chamber (Fig. 49).

90. **Effect of Carbonic Acid and Oxygen.**—With the aid of a gas-chamber (Fig. 49), and cilia from the mussel's gill or frog's pharynx, arranged as in the last experiment, the effects of various gases may be studied.

A stream of carbonic acid (supplied by the usual apparatus, Fig. 46) at first accelerates, but soon retards, and finally arrests the movement. On the substitution of a stream of oxygen or air the movement is after a time resumed.—(*Kühne.*)

91. **Effect of Alkalies.**—The addition of a very dilute solution of caustic potash accelerates the movements of cilia when these are becoming sluggish previous to their death; it may even recall the movement after it has ceased for some time.—(*Virchow.*)

For other experiments on ciliary motion see Kühne, *Schultze's Archiv*, ii. 372; Engelmann, *Journ. of Anat. and Physiol.*, iii. 420.

92. **Structure of Ciliated Epithelium** (H.)—Ciliary

motion and the structure of ciliated epithelium may be studied in cells obtained from the frog's pharynx, or from the trachea of rabbit or cat. If the latter animals be employed, they should be killed by a blow on the head, and *not by ether or chloroform*, otherwise the cilia will be paralysed. The cells may be examined fresh, and then stained with magenta to bring out the nucleus and the granular protoplasm; or they may be placed for twenty-four hours in $\frac{1}{4}$ per cent osmic acid, and then examined. The cells may be rendered easily separable by a twenty-four hours' immersion in dilute alcohol (§ 284), and then scraping the epithelial surface. Magenta then stains the cells very readily.

93. Scrape off some cells from the tracheal mucous membrane of a cat, hardened in osmic acid as above mentioned; add glycerine; tease with needles; cover, and examine. Observe the nucleus and nucleolus, the granular protoplasm sometimes with vacuoles, the cilia and the clear band under them. The clear band, however, may not now appear homogeneous, as in the fresh condition, but striated vertically.

With a power of about 1000 diam., it may be seen that the striæ are apparently continuations of the cilia into the granular protoplasm. They may be seen passing a very short way below the clear band; but we have never seen them—even after various methods of staining—passing to the nucleus, as has been alleged.

A preparation of ciliated epithelium for preservation will be obtained in the section of hardened trachea to be afterwards examined (§ 115). The cells hardened in osmic acid may be preserved in potassium acetate (§ 351) or Farrants' solution.

94. **Columnar Epithelium and "Chalice Cells"** (H.)—Remove a portion of the wall of the large intestine from a cat recently killed. Wash the mucous membrane with $\frac{3}{4}$ per cent salt solution. Scrape off the epithelium and diffuse it with the point of a needle in a drop of magenta solution. Columnar cells and chalice cells will be found.

a. Columnar cells.—These resemble ciliated cells without their cilia. At the free border of the cell there is a clear band which, unlike the granular substance of the cell, does not stain readily with magenta. Fine vertical striæ may possibly be seen in the band; these, however, will probably be more evident in the next preparation.

b. Chalice cells.—These cells are found amidst the ciliated epithelium of the respiratory passages, and the columnar epithelium of the intestine more especially. They are most readily found in the large intestine of the cat, and after staining with magenta, they may be easily seen in a preparation made as above.

They are generally about twice as broad as the ordinary columnar cells; some, however, are about the same breadth. The free end of the cell is open, with a margin that may be regular or irregular. The mouth of the cell leads into a large vacuole. A nucleus with a small quantity of protoplasm is usually found near the attached end of the cell.

95. Scrape the mucous membrane of small intestine of cat, hardened in chromic acid and potassium bichromate (§ 7). Diffuse the scraping in water with the point of a needle; cover, and examine (H).

a. Profile View of the Cells.—The ordinary columnar cells may be formed singly or in clusters. The fine vertical lines, in their striated borders, are most readily seen with a power of 1000 diam., nevertheless, they are discernible with a power of 300, in specimens that have been macerated a sufficient time. They are probably fine canals (Kölliker). A chalice cell may perhaps he found here and there between the columnar cells.

b. View of the free ends of the cells.—A superficial view of the free ends of the cells has the appearance of a mosaic. The polygonal shape of the cells results from their collateral pressure during their growth. The rounded opening of a chalice cell may be seen. This is generally smaller than the ends of the neighbouring columnar cells.

96. The mosaic and the openings of the chalice cells may be readily seen if a portion of perfectly fresh small intestine of the cat or frog be

laid open, washed in distilled water, and silvered (§ 328), the outlines of the cells are rendered dark and distinct. In the silver process in this case, use $\frac{1}{6}$ per cent solution of silver nitrate, and agitate the intestine in the solution for a minute or so.

97. The existence of mucin in the ordinary columnar cells may be thus shown. Scrape the mucous membrane of the frog's small intestine. Diffuse the scraping with a needle in a drop of water. Cover, and examine at once. From the striated ends of the cells very delicate hyaline blebs rapidly grow and become detached, their place being taken by others. These hyaline corpuscles apparently result from endosmose into the mucin within the cells.

The precise significance of chalice cells must still be regarded as doubtful. Whether they are to be regarded as unicellular glands (F. E. Schultze) for the special purpose of secreting mucus, or whether they are merely old cells undergoing mucin transformation and breaking down, is not fully shown. The fact that cells resembling them may be produced in large numbers by prolonged maceration of the intestine in chromic acid favours the latter view.

98. **Transitional Epithelium** (H.)—Examine a preparation made by scraping the mucous membrane of the bladder of cat or rabbit prepared as directed in (§ 8), and preserved in Farrants' solution. In this variety of epithelium the cells may be squamous, columnar, or quite indefinite in shape.

The various forms of *secreting* epithelium will be examined in the different glands, and other points regarding epithelium will be conveniently studied with the various organs.

CONNECTIVE TISSUES.

99. **Elastic Tissue** (H.)—Cut the *ligamentum nuchæ* of a calf or ox *vertically*, and strip off a few fibres from the cut surface with forceps. Place the tissue on a slide, add a drop of acetic acid, and tease it thoroughly with a pair of needles (§ 290, *a*). The acetic acid softens the white fibrous tissue of the ligament, and so facilitates the separation of the elastic fibres, upon which it appears to have no action. Cover, and examine.

The fibres refract the light strongly, and therefore have a distinct dark border. They branch and form an anastomosing network. Their ends may be curled or straight. The fibres under examination are a very coarse variety, being about the breadth of a blood corpuscle. In the vocal cords and in areolar tissue generally, they are much finer. This preparation may be preserved in Farrants' solution or glycerine; in the last, however, the borders of the fibres are not nearly so dark, owing to the high refractive index of the medium.

100. **Areolar Tissue** (H.)—*a.* Rapidly spread out with needles on a *dry* slide a small piece of subcutaneous tissue from a cat or rabbit. A dry slide is taken in order that the tissue may adhere to it, and so be spread out readily. The tissue must not, however, be allowed to dry. Add salt solution and examine. Notice that the white fibres consist of bundles of fibrils. The borders of the white fibres are faint and ill defined. An elastic fibre may be found here and there in the field; unlike the white fibre, its margin is sharply defined, owing to its substance being strongly refractile.

b. Remove the cover-glass and add acetic acid. It causes no change in the elastic fibres, but the white fibres swell up, the fibrils disappear, and nothing marks their position excepting a clear jelly. The nuclei of the connective tissue corpuscles are brought into view. Areolar tissue may be preserved in potassium acetate or weak spirit. Glycerine causes the white fibres to swell up and become transparent or hyaline. This preparation need not, however, be preserved, as specimens will be obtained in sections of the alimentary canal.

The splitting of the white fibres into fibrils is very easily effected after maceration for a fortnight or so in a 10 per cent solution of sodium chloride, often changed, or in lime or baryta water. (Rollett.) These agents appear to soften or dissolve a cement which unites the fibrils.

White fibrous tissue may be dissolved by boiling in water (§ 285) or dilute sulphuric acid (§ 286), and may be softened by maceration in dilute alcohol (§ 284).

101. (H.)—Examine a preparation of intermuscular

fascia from the leg, or of sub-peritoneal tissue from the lumbar region of a frog recently killed. A small portion of the tissue is excised with scissors, and gently spread it out on a slide in a drop of aqueous humour (§ 265). Observe the stationary and amœboid connective tissue corpuscles. The latter, often termed the wandering cells of connective tissue, appear to be identical with white blood corpuscles. The former are the true connective tissue corpuscles. Endeavour to see the *locomotion* of the wandering cells. The changes of shape, but *without locomotion*, of the proper connective tissue corpuscles may perhaps also be seen.

102. Connective tissue corpuscles may also be advantageously studied in the loose subcutaneous tissue of the groin of a young rabbit, cat, or guinea-pig, recently killed. The tissue should be gently spread out on the dry slide, as in § 100, and magenta added before the tissue becomes dry. This dye reveals the corpuscles with great readiness. The connective tissue corpuscles proper are usually flattened. When seen in profile they are often fusiform. Some are continuous with elastic fibres, others have no distinct processes.

103. (H.) — Examine the preparation of omentum already made (87D), and observe the connective tissue corpuscles *amidst* the fibres, and the epithelium here and there clasping the bundles of fibres. Amœboid cells (no longer moving, of course) may sometimes be seen singly or in groups here and there on the surface of the bundles.

104. **Tendon.**—Sections of fresh tendon may be readily made with the freezing microtome (§ 302); or the tissue may be hardened in $\frac{1}{4}$ per cent chromic acid, or hardened by drying, and then cut; the tendon being either simply held in the hand or imbedded in paraffin and cut in the microtome (§ 303).

Dry a tendon of an animal such as a sheep or a rabbit, until it is just *dense enough* to be readily sliced. Make an excessively thin transverse section with a scalpel or a razor. The former is preferable, as the tendon is often over-dried, and in that case apt to spoil a blade so thin as that of a razor. The knife should be wetted with a spirit, for water

does not adhere to the blade (see § 295). Probably several sections may have to be made before a sufficiently thin one is obtained. It is not necessary to have one embracing the whole thickness of the tendon, but care must be taken to have it exactly transverse and not oblique. Examine the section in water.

(L.) A tendon consists for the most part of bundles of fibres running longitudinally; a few transverse fibres encircle the longitudinal bundles, forming a sheath for the whole tendon. This sheath sends inwards branching trabeculæ between the bundles, so as to divide the tendon into compartments. These trabeculæ and the cut ends of the enclosed longitudinal bundles are readily seen with L.

(H.) Observe the compressed branching spaces between the transverse sections of the longitudinal fibres. Some of the spaces contain nucleated corpuscles. (This preparation need not be preserved.)

If such a section be placed in glycerine, the fibres swell up and become hyaline. The cut ends of elastic fibres may then be seen within the longitudinal bundles and the nuclei of the cells in the branching, spaces may also be seen.

105. Examine T. S. tendon hardened in chromic or picric acid, and preserved in glycerine. In this, the spaces between the bundles are not so compressed as in the dried tendon.

106. Examine a tendon from the tail of a rat prepared as follows. Divide the skin about the middle of the tail of a young rat or mouse just killed. Forcibly detach the extremity of the tail, so as to rupture the caudal vertebræ, and pull out the bundles of fine tendons with which these are surrounded. Two methods may then be adopted—1. They may be placed in $\frac{1}{2}$ per cent solution of chloride of gold for two or three minutes, and then exposed to the light in water acidulated with a few drops of acetic acid (see § 330). The tendons are then teased with needles, and mounted in Farrants' solution or in glycerine. 2. The tendons may be placed for a minute or two in water slightly acidulated with acetic acid, to cause them to swell up and become transparent. They are then washed in water stained with log-

wood (§ 323) and mounted in Farrants' solution, or in glycerine.

Rows of flattened cells (Ranvier's cells) will be seen lying between the longitudinal bundles of fibres. The cells are somewhat quadrangular plates consisting of a distinct nucleus surrounded by granular protoplasm. Their relation to the fibres has been the subject of considerable dispute. In a piece of tendon stained with gold and successfully teased with needles, one may see, as Boll first pointed out (*Schultze's Archiv*, 1871, p. 281), that the cells clasp the bundles and apparently extend about half-way round them. A longitudinal stripe in the row of cells was pointed out by Boll, and termed by him the "elastic stripe." More than one stripe may be seen in each row. A view in full face of the margin of a cell has the appearance resembling Boll's stripe. Ranvier has shown, however (*Traité Technique d'Histologie*, p. 352), that Boll's stripes are due to ridges on the cells; such a ridge as that seen on a bent tile or plate of zinc on the top of a house.

107. For the demonstration of these ridges and the isolation of tendon cells, Ranvier (*loc. cit.*) recommends the following:—Place a tendon for twenty-four hours in 1 per cent osmic acid, and then for the same period in picro-carmine. Endeavour to isolate the tendon cells by teasing with needles, and examine in glycerine containing 1 per cent formic acid.

108. **Adipose Tissue** (H).—Examine the preparation of omentum of guinea-pig or cat already made (§ 87D). Observe the shapes of the fat cells when they are isolated, and when they are pressed one against another. The nucleus of the fat cell may have been stained by the silver, and if so, it will be readily seen, especially if a profile view be obtained. The nucleus is also rendered evident by carmine or logwood staining. Each cell consists of a thin membrane more or less filled by a drop of fat, having a nucleus with a small quantity of protoplasm in contact with the membrane at one part of the cell. Fat cells when silvered and mounted in Farrants' solution or in glycerine are very perfectly preserved.

109. The envelope of the fat cell may be readily shown by the

old method of placing in ether for twenty-four hours a piece of adipose tissue from an animal just killed. The fat is thus abstracted more or less completely from some of the cells, and the collapsed membranes are rendered visible. Perhaps the best method, however, is the following :—Digest a piece of skin as described in § 223, *b ;* make vertical sections, stain with carmine, and preserve in glycerine. In many of the fat cells there is a wide space between the fat and the envelope, and the stained nucleus is readily seen on the interior of the latter.

109A. Examine a preparation of fat cells showing crystals of margarine. These sometimes appear after death. They are almost always to be found in fat taken from an animal recently killed, and mounted for some time in glycerine. They are also often found in the fat cells of organs, *e.g.*, skin hardened in chromic acid and mounted in glycerine.

110. **Adenoid Tissue** (H.)—With the aid of the freezing microtome make an extremely thin section of a fresh mesenteric gland of a fasting animal recently killed. Detach the lymph corpuscles from the adenoid tissue by gently shaking the slice in a test-tube with $\frac{3}{4}$ per cent salt solution ; mount the section in Farrants' solution, and examine.

Adenoid tissue consists of a reticulum of nucleated connective tissue corpuscles, the processes of which have been transformed into fibres. Lymph corpuscles will be found in the interspaces.—(*This section will be wanted on a future day.*)

111. Examine a section of a lymph gland prepared as follows :—Into the mesenteric gland of a fasting cat or dog recently killed, thrust the nozzle of a Wood's syringe through the capsule of the gland, and inject $\frac{1}{4}$ per cent silver nitrate solution. Make sections with the freezing microtome, wash away the superfluous silver with distilled water, expose in the water to diffuse daylight for a few hours, stain with logwood. The adenoid tissue is coloured brown, while the lymph corpuscles are rendered violet. The latter may be detached by shaking in a test-tube with ordinary water. The sections may be preserved in Farrants' solution, or in glycerine, or glycerine jelly.

Other preparations of adenoid tissue will be obtained

on a future occasion in sections of the lung, intestine, and spleen.

112. **Mucous Tissue** (H.)—Examine a preparation of vitreous humour, made by mounting a small piece of the vitreous humour of the kitten before birth, in Farrants' solution. Connective tissue corpuscles are seen in a jelly-like matrix.

CARTILAGE.

113. **White Fibro-Cartilage** (H.)—Make a section with scissors of fresh white fibro-cartilage from the peripheral part of the intervertebral disc of an ox or sheep after hardening for forty-eight hours in a saturated solution of picric acid. Tease with needles, and preserve in Farrants' solution or in glycerine.

The matrix consists of very delicate fibrils like those of white fibrous tissue. Sometimes, however, it is so difficult to see the fibrillation that one is apt to suppose that the matrix is homogeneous. The cells consist of a nucleated protoplast enclosed in a capsule, resembling that found around the protoplasts of hyaline cartilage. The cells may be found in various stages of fission. Their characters are much more clearly seen with a power of 800.

114. **Yellow Fibro-Cartilage** (H.)—Place the epiglottis of a sheep for a day in rectified spirit: nothing else hardens this so well. Make sections with a razor. Examine, and preserve in Farrants' solution or in glycerine.

This tissue consists of nucleated cells surrounded by a matrix, which, in the greater part of the cartilage, consists of a spongy material apparently of the same nature as elastic tissue. Towards the periphery of the cartilage there are distinct yellow elastic fibres in the matrix. But the greater part consists of a yellow spongy substance, the fine spicules of which when divided transversely resemble granules; hence the erroneous statement that the matrix of this tissue consists of elastic fibres imbedded in a granular material. It is advantageous to examine this tissue with a power of about 800.

115. **Hyaline Cartilage,** *Trachea*—T. S. tracheal

rings of cat, hardened in $\frac{1}{4}$ per cent chromic acid (§ 6). The specimen will be a valuable one if the section be made with the freezing microtome; for in that case the ciliated epithelium lining the trachea will not be destroyed, as is apt to be the case when such a tissue is imbedded in paraffin. Mount the section in Farrants' solution or in glycerine, and examine.

(L.) The position of the cartilage, epithelium, and the tracheal glands.

(H.) *a. The cartilage*, consisting of cells in a hyaline matrix. Observe the nucleus and granular protoplasm of which the cells consist; the distribution of the cells in the matrix; the groups result from recent proliferation of the cells.

b. The perichondrium, consisting of white fibrous tissue. Notice the gradual transition between the matrix of the cartilage and the fibres of the perichondrium on the one hand, and between the cells of the cartilage and those of the perichondrium on the other. The latter, however, are difficult to discern unless they are stained.

c. The ciliated epithelium.—The characters of the ciliated cell have been already studied. The cells are here seen *in situ*. There are several layers of them, but only the upper layer (those next the cavity of the trachea) are ciliated. The others are of irregular shape, and are apparently young cells that are destined to develop into those with cilia.

d. The glands, cut in all directions, will be found inside the cartilage. They are lined by epithelium, and their ducts may perhaps be traced to the inner aspect of the membrane on which they pour their mucous secretion.

116. *Costal Cartilage* of human adult kept for twenty-four or forty-eight hours in a saturated solution of picric acid. With a razor make a thin T. S. through a point of incipient ossification. Mount in Farrants' solution or in glycerine, and examine.

(H.) The diffuse yellow colour resulting from the picric acid might be removed by immersion in water or in alcohol, but it is advantageous to retain the colour in this case. At the point of incipient ossification groups of proliferating

cartilage cells will be found, and a clear capsule will be seen around most of the protoplasts—thickest in the largest ones. The appearance reminds one of the capsule enclosing each protoplast in white fibro-cartilage. The diffuse picric acid staining is of much service in rendering these capsules evident. The matrix of hyaline cartilage apparently consists of these capsules pushed outwards and matted together by new ones that are secreted by the protoplasts proliferating by fission within the old ones. Side by side with the proliferation of the cartilage cells, observe the fibrillation of the matrix. This consists in the appearance of great numbers of calcified fibrils running parallel one with another, and usually with the long axis of the groups of proliferating cells. The proliferation of the cells and the calcified fibrillation of the matrix are the two earliest stages of intra-cartilaginous ossification, which will be further studied in another preparation (§ 125). If this preparation happen to be the costal cartilage of an old subject, cartilage cells in a state of fatty degeneration may be found here and there. The protoplasm becomes replaced by fatty particles.

117. Staining Agents for Hyaline Cartilage.—*a. Osmic acid.* The fatty particles that occur sparingly in most cartilage cells, and often so abundantly in those of an old subject, may be readily demonstrated by placing the slices in $\frac{1}{4}$ per cent osmic acid for 12 to 24 hours. The fat is blackened by the acid.

b. Chloride of gold is very useful for staining the cells. It renders them violet, and it has the great advantage of not causing any retraction of the protoplasm from the matrix. Vertical or longitudinal sections of the articular cartilage of the femur of a frog, recently killed, when stained with gold give readily the characteristic appearance. It should be kept in the gold solution twenty minutes, and otherwise treated as described in § 330.

c. Silver Nitrate stains the matrix of hyaline cartilage, and is therefore of value in tracing its relation to the protoplasts. Ranvier's method is to wash the head of the frog's femur in distilled water for a few seconds; place it in $\frac{1}{3}$ per cent solution of silver nitrate until it becomes opaque; wash in distilled water for some minutes: remove thin slices from the articular surface; place them in glycerine on a slide, and expose to the light till the matrix has become sufficiently dark. Or the articular surface may be shaved off, and the sections placed at once in the silver solution.

d. The value of *picric acid* has been alluded to in § 116.

BONE.

118. **Method of preparing Unsoftened Bone.**—The bone is cut into tolerably small pieces, and freed from its soft parts by maceration for months in water changed from time to time. The bone must not be allowed to become dry previous to maceration, otherwise the bony tissue may become indelibly marked with greasy stains. In the case of a long bone, much of the fat may be rapidly detached from the medullary canal and cancellated tissue by connecting it to a water-tap by an elastic tube. The bone is then dried, and if it contain much spongy tissue, it is impregnated with a strong solution of gum, hardened for a day in methylated spirit, dried, and then cut with a fine saw in any desirable direction. The spongy tissue is supported by the gum, and thus rendered less liable to be fractured by the saw. The sections are ground sufficiently thin by rubbing them on an ordinary hone, moistened with methylated spirit to retain the gum *in situ*. They are then placed in water to remove the gum, thoroughly washed in a stream of water, and finally dried.—(*Ranvier.*) They are commonly mounted dry, that is to say, in air. They may also be mounted in dammar (§ 347, *c*); this is apt to render them too transparent.

119. **Method of preparing Softened Bone.**—The soft parts of bone may be successfully hardened and preserved, while the calcareous matter is removed, by maceration in $\frac{1}{2}$ per cent chromic acid, with the addition of 1 per cent nitric acid, after the method described in § 15. When the decalcified bone is transferred to rectified spirit, it becomes of a greenish tint, owing to the production of chromium sesquioxide (chromic oxide). The softened bone may be readily sliced with a knife, or torn into shreds for the demonstration of its lamellæ.

Ranvier recommends for the softening of bone a saturated solution of picric acid, and this, for fœtal bone, is perhaps the best agent. The bone should be divided into very small pieces and placed in a large quantity of the fluid, kept saturated by the presence of an excess of the crystals. When softened, the picric acid may be partially or completely removed by immersion in water or spirit. It should be wholly removed only when the preparations are to be stained, *e.g.*, in picro-carmine.

120. **Staining Agents for Softened Bone.**—*a.* The greenish coloration of the matrix by *chromic oxide* has been alluded to in § 119.

b. Picro-carmine (§ 322) gives fair results. It stains the bone corpuscles, and to a less extent the matrix. Before staining with this agent all chromic or picric acid should be removed by washing the sections in water.

c. Purpurine is strongly recommended by Ranvier as a staining agent for the bone corpuscles, on account of its staining *them* only. Chromic or picric acid is previously washed out of the sections, and they are then placed in a solution of purpurine (§ 326) for twenty-four to forty-eight hours. It does not, however, appear to show more than picro-carmine does.

Sections of softened bone unstained should be mounted in rectified spirit or glycerine jelly (§ 350); glycerine renders them too transparent. If stained, they may be mounted in Farrants' solution, glycerine, or glycerine jelly.

121. **Structure of Bone.**—Examine a T. S. unsoftened bone prepared as directed in § 118.

(L.) The compact and spongy bone. The Haversian systems in the former, with a Haversian canal cut transversely in or near the centre of each system. The lacunæ with their canaliculi.

(H.) The lacunæ usually more or less flattened. The canaliculi dividing and anastomosing with those of neighbouring lacunæ, so as to form what is probably a system of lymphatic channels. The canaliculi of the lacunæ nearest the Haversian canal open into it, and thus the lymph can readily pass from the blood capillaries in the canal into the canaliculi. The contents of the Haversian canals and lacunæ will be found in preparations of decalcified bone.

122. Examine a V. S. unsoftened long bone. (L.) The Haversian canals mostly cut vertically. They anastomose, and some may be found opening on the periosteal surface. Others may be found passing into the spaces in the spongy bone. The Haversian systems being divided vertically, the concentric arrangement of their lacunæ is of course not here seen.

123. T. S. softened long bone of cat. If the section is unstained, examine it in dilute alcohol (§ 284)—for the

cover-glass requires to be removed—and finally mount in glycerine jelly; glycerine renders the bone corpuscles too transparent. If, however, the section be stained with picro-carmine, examine, and preserve it in Farrants' solution, glycerine, or glycerine jelly.

(L.) *The compact and spongy bone.*—If the section be through the central part of the shaft of the bone, little cancellated tissue will be seen. The Haversian systems—each consisting of a Haversian canal, with a concentric series of lamellæ—are seen divided transversely. The systems are of somewhat irregular shape, and the Haversian canal is often placed eccentrically. This results from the substance of the system growing from the Haversian canal outwards, most rapidly in the direction of least resistance. Under the periosteum the lamellæ have a direction parallel with that membrane. Between the Haversian systems there are small sets of lamellæ, that are generally more or less parallel with the direction of the sub-periosteal lamellæ. In the development of the bone the lamellæ produced around the Haversian canal grow outwards, and cause the partial, and in some places the complete, absorption of those lamellæ parallel with the periosteum, and originally produced from it. The lamellæ and the bone corpuscles will, however, be more clearly seen with H.

(H.) *The bone corpuscles.*—Nucleated masses of protoplasm in branching spaces, the lacunæ and canaliculi. The last are difficult to see in softened bone, unless it be stained with picro-carmine. The wall of the lacunæ and canaliculi consists of a homogeneous calcified membrane that may be detached from the lamellæ, although with difficulty—if softened bone be teased with needles. (*Virchow.*)

b. The lamellæ.—Examine them in a Haversian system. Five or six lamellæ usually intervene between two neighbouring lacunæ. The elliptical shape of the lacunæ apparently results from compression between the lamellæ.

c. Sharpey's fibres may be found perforating the sub-periosteal lamellæ. They are calcified fibres that run from the periosteum inwards between the Haversian systems. They are not found *within* the latter. Remove the cover-

glass, and tear off a few of the peripheric lamellæ. After this some of Sharpey's fibres may be found isolated, by being torn out of their sockets.

d. The periosteum.—In the *adult* animal the periosteum consists simply of fibrous tissue, with a connective tissue corpuscle here and there imbedded amidst the fibres, or in contact with the bone, but they are not readily seen unless the section be exceedingly thin and stained. In another preparation it will be seen that the periosteum of the young bone has a very different appearance. The contents of the cancelli and of the Haversian canals will also be better seen in other preparations.

124. **Development of Bone.** — *Young periosteum.* (H.)—Examine a T. S. long bone from kitten at birth, softened in the chromic and nitric fluid, or in picric acid, and stained with picro-carmine. A layer of nucleated cells (osteoblasts) is found under the fibrous tissue of the periosteum close to the bone. On carefully examining the whole thickness of the periosteum, it may be seen that these cells are proliferations from connective tissue corpuscles. They may be traced into the Haversian canals, and through these into the cancelli. Everywhere they are in contact with the bone. These osteoblasts ultimately become the bone cells; the matrix of the bone grows around and encloses them. The manner in which the canaliculi are formed is disputed; some maintaining that they are mere spaces left in the bony matrix during its growth. It may be seen, however, with (H.), and still more clearly with a power of 1000 diam., that the osteoblasts have often very distinct processes before they are included in the bone. This preparation shows a very delicate fibrous tissue in the cancelli and in the Haversian canals, through which it is continuous with the periosteum, capillaries and larger vessels may also be found in the cancelli, Haversian canals, and in the periosteum, especially in its inner layer.

125. **Intra-cartilaginous Formation of Bone.**—V. S. bone and articular cartilage from softened bone of adult cat, prepared as directed in § 15. Treat in the same manner as the softened bone (§ 123).

(L.) Examine cancelli, articular cartilage, and a layer of calcified cartilage close to the bone.

(H.) *a. Osteoblasts.*—In the cancelli osteoblasts will be found close to the bone. Observe their irregular shape, some with distinct processes, and all of them having a bright refractive appearance. The other cells found in marrow will be examined in a preparation from fresh bone.

b. Articular cartilage.—Near the articular surface the cells are flattened; about the middle of the cartilage they are in irregular groups; near, and in the calcified layer, they are arranged in rows, owing to repeated proliferation of the cartilage cell by cleavage at right angles to the long axis of the row. The lime has of course been removed from the matrix in the calcified zone, but in specimens not fully decalcified, a fibrillation of the matrix similar to that seen in the rib (§ 116) may be recognised. On very carefully examining the line of junction between the cartilage and the bone, one can here and there see a cartilage protoplast becoming surrounded by the matrix of the bone that grows up around it. The production of a bone cell from a cartilage cell may be thus demonstrated; but this mode of bone formation seen *above* the epiphysis in an *adult* animal must be regarded as exceptional, and differing decidedly from the mode discernible under the epiphysis in a young animal.

126. The intra-cartilaginous development of bone may be very clearly studied in a V. S. of a long bone or scapula of a kitten at birth, and in a V. S. of the phalanx of a human fœtus at the fourth month (doubtless the phalanx of a kitten would show the same, but this we happen not to have examined). The bones should be softened in picric acid, and the former may be unstained while the latter is stained with picro-carmine. It is advantageous to inject the blood-vessels with soluble Prussian blue (§ 333) previous to softening.

127. The vertical section of the phalanx, prepared as above, is exceedingly instructive.

(L.) The ossification begins in the middle of the shaft, and then extends towards the ends. A wedge-shaped piece

of bone may be seen growing inwards from the perichondrium to meet its fellow from the opposite side. Between the two there are irregular *medullary spaces* filled with cells. Blood-vessels extend into them from the perichondrium through canals in the bone. Rows of cells may be seen in the cartilage near the medullary spaces.

(H.) The proliferation of the cartilage cells close to the medullary spaces may be readily seen. A single cavity contains a number of cells, and these cavities finally become continuous with the medullary spaces into which blood-vessels have grown from the perichondrium. The cartilage matrix is entirely absorbed, as has long been known, but whether the cartilage cells become marrow cells or osteoblasts, or what becomes of them, it would be difficult to say, for one may detect something very like the diapedesis of white blood corpuscles through the walls of the capillaries, and the emigrated blood corpuscles may play some part in the process of forming osteoblasts, as has been suggested by Gegenbaur and others.

128. (H.)—A V. S. of a somewhat older bone of a kitten, prepared as above, shows the rows of cartilage cells, the medullary spaces with their capillaries, and cells around them. The fate of the plates of cartilage matrix between the medullary spaces may be clearly traced.

The cartilage matrix becomes encrusted with the matrix of the future bone, and slowly disappears. Osteoblasts are gradually enclosed in the bony matrix, and thus become bone cells. It would be difficult to say what is the precise genetic relation between the osteoblasts and the bony matrix, for a thin layer of the matrix may be seen extending up towards the cartilaginous ends of the medullary spaces, without any appearance of osteoblasts in it, in the first instance. The exact mode of origin of the osteoblasts in intra-cartilaginous formation of bone, must therefore still be held as doubtful.

129. **Marrow** (H.)—As yellow marrow consists chiefly of fat cells, it need not here be alluded to. *Red* marrow, however, is worthy of careful study. Take a rib from a rabbit, cat, or dog, not fully grown; divide it transversely,

and then with a common knife remove a slice of the spongy tissue. Place it on a slide in a drop of salt solution. With needles detach the bony spicules from the marrow and throw them away. Cover, and examine.

Most of the cells in the preparation are the proper *marrow cells* of Kölliker. These somewhat resemble white blood corpuscles, but when acted on by dilute alcohol or acetic acid, they usually exhibit a single nucleus, and often also yellowish particles. Large multi-nucleated masses of protoplasm, commonly termed *giant cells* (the *myeloplaxes* of Robin), may be found here and there. They vary in size from five to twenty times that of a blood corpuscle. In a section of the cancelli of decalcified bone of a somewhat young subject, a giant cell may be found in contact with the osseous tissue here and there amongst the osteoblasts. Believing that they are concerned in the absorption of the neighbouring bone—which often appears as if scooped out—Kölliker has named them *osteoclasts.*

Neumann and Bizzozero have advanced the opinion (*Centralblatt*, 1868, pp. 689, 855, and 1869, p. 149), that on account of the presence of nucleated red blood corpuscles, similar to those of the embryo, red marrow is to be regarded as a blood gland. We have entirely failed to find such corpuscles; but, on the other hand, one can sometimes see a group of red corpuscles enveloped in a mass of clear colourless colloid material, similar to the bodies found in the spleen, and which are there regarded as evidence of the *disintegration* of red corpuscles.

TOOTH.

130. Sections of unsoftened tooth are prepared according to the same method as bone (§ 118). Owing to the absence of fat from the tooth pulp, however, the maceration is soon accomplished. The method for softening tooth is the same as that adopted for bone (§ 119). The enamel, however, disappears, owing to its very large percentage of calcareous matter.

131. **Structure of Unsoftened Tooth.** V. S. Unsoftened Tooth. (L.)—Observe the pulp cavity, the dentine, the enamel outside the dentine in the crown, and the crusta petrosa outside the dentine in the fang of the tooth. The

wavy lines running from within outwards in the dentine are the dentinal tubules. The arched *incremental* lines which may sometimes be seen crossing the dentinal tubules in the crown of the tooth indicate a lamination of the dentine apparently due to incomplete calcification of its matrix during development. The enamel consists of prisms set with their ends on the dentine. Lines more or less concentric, and of a brown colour, may often be seen crossing the enamel prisms. Their cause is uncertain, but they look as if they resulted from the deposit of a brown pigment.

(H.) The enamel prisms are arranged in bundles that often decussate at their inner ends near the dentine. In the preparation there may be some of these bundles cut transversely. These show the fibres to be more or less hexagonal. The dentinal tubules have primary dichotomous divisions, and give off great numbers of fine lateral branches. The latter are usually very evident in the outer part of the dentine of the fang of the tooth. In the crown, very probably, minute rounded apertures in the transverse sections of the dentinal tubules may be found. These are, however, most easily seen in a V. S. of dentine made at one side of the pulp cavity. *Interglobular spaces* may perhaps be seen in the outer part of the dentine. These are irregular cavities, which, like the incremental lines that frequently open into them, apparently result from defective calcification. In such a preparation as this they have a black appearance, resulting from their being filled with débris during the grinding of the tooth. The *crusta petrosa* is merely bone.

132. **Structure of Softened Tooth.** V. S. jaw of cat softened in dilute chromic and nitric acid, as directed in § 15. Mount in Farrants' solution or in glycerine jelly. The latter is preferable.

(L.) The tooth in the alveolus. The periodontal membrane serving as a periosteum for the crusta petrosa and the interior of the alveolus. If the section be one of the *lower* jaw, the canal containing the inferior dental nerve and blood-vessels will be cut across, and good sections of these structures thus obtained. All the parts of the tooth

are present except the enamel, and probably also much of the pulp will be wanting.

(H.) The dentinal tubules will be readily seen. Carefully examine the pulp close to the dentine for *odontoblasts*. These are nucleated cells that send one or more somewhat stiff processes from their outer aspects into the dentinal tubules, constituting the *dentinal fibres*, or fibres of Tomes. The odontoblasts are, however, very liable to be lost during the softening of the tooth. Nerves and blood-vessels and a delicate connective tissue are also found in the pulp, but these need not be looked for in this preparation.

133. The structure of the pulp of the full-grown tooth can only be investigated by breaking the tooth across with a hammer. The broken tooth should then be hardened for twenty-four hours in $\frac{1}{6}$ per cent osmic acid.

134. The odontoblasts and fibres of Tomes may be seen in sections of the teeth *in situ*, when they are very young and still within the dental sacs.

135. The dentinal tubules may be isolated by boiling a softened tooth for about ten minutes in 1 per cent sulphuric acid. The tubules appear to be calcified membranes, imbedded in a calcified tissue somewhat similar to that of bone. The latter disappears when boiled in the dilute acid.

MUSCLE.

136. **Non-Striped Muscle.**—*Methods of Preparation.* —*a.* Kölliker's method for the isolation of the fibres consists in placing a piece of fresh intestine or stomach in dilute nitric acid (acid one part, water four parts) for twenty-four or thirty-six hours. The muscular fibres are hardened and assume a yellow colour, while the fibrous tissue that binds them together is softened and dissolved. The acid is then removed by washing in water. The nucleus in each fibre may be clearly brought into view by logwood staining. The fibres are separated by teasing.

b. The fibres may also be separated by teasing after maceration for two days or so in dilute alcohol (§ 284).

c. The fibres may also be prepared by the gold method. For this purpose portions of the muscular coat, from the small intestine of a rabbit or other animal recently killed,

are placed in gold chloride solution, and treated as directed in § 330. The nucleus of the fibre is stained.

d. The nuclei of the fibres may also be well stained with picro-carmine. A piece of frog's bladder when treated with this dye (§ 322) gives beautiful preparations.

137. **Structure of Non-striped Muscle.**—Strip off a small piece of the muscular coat of the intestine, hardened in dilute nitric acid as above described. Tease it with needles in a drop of Farrants' solution; cover, and examine.

Each fibre is a spindle consisting of a nucleated band of sarcous substance, without an envelope. The whole fibre is evidently an elongated cell, the protoplasm of which has been transformed into a substance, that—like the substance of a cilium—contracts in a definite direction.

138. Examine a preparation of frog's bladder, carminised. Notice the spindle-shaped and the *triradiate* fibres. *The latter might, by the inexperienced observer, be mistaken for nerve cells.*

Excellent preparations of the fasciculi of non-striped muscle, divided transversely and longitudinally, will be found in the sections of stomach and intestine at a future time.

139. **Striped Muscle.** — *The Sarcolemma* (H).—*a.* Decapitate a frog, and isolate one of its muscles; the sartorius is to be preferred, because its fibres are parallel and easily separated. Make a vertical section of the muscle with scissors, and tease the fibres in a drop of water, in order to study the sarcolemma. Look for the sarcolemma raised from the sarcous substance, by imbibition, in the form of a vesicle, or stretched between the ends of fibres broken across, or prolonged as a funnel-shaped process from the end of a ruptured fibre.

b. Effect of acetic acid.—Remove the preparation from under the lens, raise the cover and add a drop of dilute acetic acid. It renders the sarcous substance very transparent, and reveals the nuclei of the sarcolemma. In mammals the nuclei are only found under the sarcolemma. In the frog's muscle they are also found amidst the sarcous substance.

Each striped fibre of the voluntary muscles is an elongated spindle developed from a cell. The sarcolemma may be regarded as the cell membrane; the nuclei inside the sarcolemma are proliferations from the original nucleus, and the sarcous substance is a modification of the original protoplasm.

140. **The Sarcous Substance** (H.)—To soften the connective tissue between the fibres, and permit of their ready dissociation with needles, prepare muscle as follows. Take a narrow strip of the sartorius of a rabbit just killed; tie a thread round both ends; fix the threads to a piece of wood with the muscle gently stretched; then place in dilute alcohol (§ 284) for two days or so. Tease a small piece with needles in Farrants' solution; cover, examine, and preserve.

Each fibre is seen to be crossed by alternate light and dim stripes. By altering the focus it may be seen that the stripes are not superficial, but extend through the whole thickness of the fibre.

141. **Cleavage of the Sarcous Substance** (H.)—*a.* With a razor make a thin V. S. of striped muscle of cat or rabbit hardened in $\frac{1}{4}$ per cent chromic acid (§ 11) to facilitate the cleavage of the sarcous substance. Tease with needles in a drop of Farrants' solution; cover, and examine.

The fibre cleaves most readily in a longitudinal direction. The result of such cleavage is a bundle of *fibrils*, each marked with a light and dim transverse stripe like the original fibre. A very fine dark line may—with a power of about 1000—be seen crossing the light stripe transversely, and dividing it into two equal portions. If the fibre or fibrils cleave transversely, the rupture takes place through this faint dark line in the clear stripe.

b. Remove the slide from under the lens, and forcibly press down the cover-glass with the *handle* of a knife or needle, and examine again. The pressure often causes the fibres and fibrils to cleave transversely. When a *fibre* cleaves transversely, it gives rise to a number of discs. The transverse cleavage of a *fibril* produces a number of

oblong particles, which have been conveniently named *sarcous prisms*. A *fibril* consists of these prisms united end to end; a *disc* is formed by their transverse union.

If striped muscle be partially digested in such a digestive fluid as that mentioned in § 223 *b*, it, as has long been known, becomes very apt to split into discs.

142. Examine, with a power of 800 or 1000 diam., a preparation of the muscle of a bee, hardened in $\frac{1}{6}$ per cent chromic acid, and mounted in glycerine. The sarcous prisms are beautifully seen. The house-fly and the water-beetle serve equally well for making such specimens.

143. Amputate the leg of a water-beetle (*Dytiscus marginalis*). With a knife slit open the chitinous case; carefully pick out the muscle; make a vertical section with scissors; add a drop of salt solution, or, still better, aqueous humour; gently separate the fibres with needles; cover, and examine without delay. (H.) Observe the contraction waves sometimes implicating the whole thickness, sometimes only a part of the fibre. The stripes are easily seen. Faint dark lines are seen crossing the dim stripe at right angles to it. These are the lines through which longitudinal cleavage of the fibre takes place.

When magnified 1000 diam. or so, the fine longitudinal lines crossing the broad dim stripe at right angles may be traced into the clear stripe on each side, near the middle of which each line becomes enlarged and forms a knob-like extremity. The whole has been compared by Mr. Schaefer to a dumbbell with a very slender shaft. He has termed these structures "muscle rods." The heads of the rods form the faint dark line in the dim stripe. The discussion regarding the cause of the stripes of muscle cannot be entered into here; but it may be safely said that the whole question is still very obscure.

144. Examine (H.) striped muscle stained with carmine. The nuclei are stained, but not the sarcous substance or the sarcolemma.

145. Examine (H.) a T. S. of striped muscle, hardened in chromic acid. Such a section as this will be obtained in a T. S. of the tongue (§ 185). The fibres are seen to be polygonal, owing to collateral pressure. In the living muscle they are rounded.

Transverse sections of fresh muscle may be readily made with the freezing microtome.

146. Examine (L.) an L. S. of muscle with its blood-vessels injected. The capillaries are seen to run mostly parallel with the fibres. These longitudinal vessels are united by short transverse branches.

147. With regard to the examination of muscle by polarised light (§ 53), the memoirs by Brücke (Stricker's *Histology*, vol. i. p. 235) and Schaefer ("Minute structure of the Leg-Muscles of the Water-Beetle," *Philosophical Trans.*, 1873) may be consulted.

NERVE TISSUE.

NERVE FIBRES.

148. **White Nerve Fibres** (H.)—Place the lumbar or sciatic nerves of a frog in $\frac{1}{6}$ per cent osmic acid for two days. Wash in distilled water for a day. Tease with needles, and preserve in Farrants' solution.

Observe in each fibre a central somewhat clear band—the *axial cylinder*. At the broken end of a fibre this band may be found projecting. It is apparently homogeneous, but in the spinal cord it may be seen that at the junction of the axial cylinder with a nerve cell it is fibrillated. Therefore the axis is believed to be a bundle of nerve fibrils held together by an interstitial cement. Outside the axis there is the *medullary sheath*, or *white substance of Schwann* darkened by the osmic acid (for convenience we term this the *white* sheath). In the cerebro-spinal nerves—but not in the brain and spinal cord—the medullary sheath is enclosed in a delicate homogeneous membrane, the *primitive sheath* of Schwann (for convenience we term this the *gray* sheath). It is difficult to see this, but it may perhaps be found prolonged from the broken end of a fibre. On tracing the fibres lengthwise, Ranvier's nodes will readily be found. Each node is a slight constriction, at which the white sheath is deficient. The axial cylinder, however, extends through the node, so that the continuity of this—the essential part of a nerve fibre—is intact. These nodes—which are only to be found

in the cerebro-spinal *nerves*, and not in the centres—indicate the division of the nerve fibre lengthwise into a number of segments, each segment being apparently a transformed cell. The gray sheath represents the cell membrane, in which a single nucleus is to be found in each segment, while the axial cylinder and white substance may be regarded as modified cell contents.

149. The nuclei of the gray sheath are best brought into view by silver staining. Place the sciatic nerve of a frog, or the intercostal nerves of a rabbit, in $\frac{1}{2}$ per cent solution of silver nitrate for two or three minutes, then treat in the ordinary way (§ 328). The nuclei are coloured brown; another very characteristic appearance, however, is produced, viz. that of small brown crosses in the fibres. Each cross results from the silver penetrating the axial cylinder and staining it, together with the cement that covers it, at the node where the medullary sheath is wanting. In such a preparation, also, the simple layer of squamous epithelium that covers the trunk of every cerebro-spinal nerve may be seen, the epithelial outlines being silvered.

150. Remove a portion of a lumbar or sciatic nerve of a frog just killed. Fray out one end of the nerve on a slide with the point of a needle in a drop of salt solution or aqueous humour. Cover the *frayed portion only*, and examine. (H.) There is a *double contour*, that is to say, a pair of lines on either side of the centre. The axial cylinder is placed between the two pairs of lines, while the interval between the outer and inner line of each pair is occupied by the white and the gray sheaths. The gray sheath may sometimes be found uniting the two ruptured portions of a fibre, or stretching as an infundibuliform process from a torn extremity. A double contour is not perceptible in a living medullated fibre. Its appearance is probably due to coagulation.

If the fresh nerve be frayed out in water, the white substance coagulates, and often assumes a fibrillated appearance.

151. Make a thin L. S. with scissors of the white matter of fresh spinal cord of sheep or other animal. An

extremely small piece must be taken. Place it on a slide, add no fluid, gently press down the cover-glass, and examine the fibres. As the white fibres in the brain and spinal cord are not surrounded by a gray sheath, they readily yield to the influence of pressure, becoming ampullated, and even broken into pieces. The fragments assume a rounded form, each one being marked like the original fibre *by a double contour*. This serves to distinguish them from nerve cells, for which they are apt to be mistaken by the inexperienced observer.

152. T. S. sciatic nerve of rabbit or cat hardened in chromic acid (§ 14). Stain with logwood (§ 323) by placing the section in a large drop of logwood solution on a slide. After staining and washing in water, remove the water by immersing first in methylated spirit in a watch glass, and then in absolute alcohol for two or three minutes. Lay the section on a slide, allow it to *partially* dry (let it just become glazed and sodden), then *insinuate* a drop of clove oil *under* the section with a sable-hair brush (camel-hair is not stiff enough). Allow the oil to *rise* through the section, *and so drive away the spirit*. The principle of this method is explained in § 347A.

(L.) Watch the clarifying effect of the oil (the section being still uncovered, of course). Finish the clarification by touching the upper surface of the section with the oil. Lastly, cover with a drop of dammar or Canada balsam solution, put on the cover-glass, and gently press it down. Water and spirit are not miscible with dammar. The spirit replaces the water, the clove oil replaces the spirit, increases the transparency of the tissue, and is miscible with dammar or Canada balsam dissolved in benzole or chloroform.

(H.) In the section prepared and mounted as above, divided nerve fibres of various sizes are seen, each with a violet centre—the axial cylinder—surrounded by the medullary substance unstained. Observe the connective tissue (*neurilemma*, *perineurium*) forming a sheath to the nerve, and sending processes between the fasciculi of the nerve fibres.

153. *Gray nerve fibres.* (H.)—Examine a preparation

of these made as follows:—Place the cervical sympathetic of a rabbit in $\frac{1}{4}$ per cent chromic acid for two or three days. Tease a small piece with needles, stain with logwood, and mount in glycerine. A spindle-shaped nucleus brightly stained can be seen here and there in the course of a fibre. The nuclei appear to belong to the gray sheath enclosing the nerve fibrils.

154. Examine (H.) a preparation of cornea, showing primitive nerve fibrils stained with gold, as directed in § 232, *b*. A network of fibrils is seen. The fibrils have usually a beaded appearance. A power of 800 or 1000 diam. is advantageous for their examination.

CENTRAL NERVE TERMINATIONS.

155. **Nerve Cells** (H.)—Make a section with scissors of the Gasserian ganglion of a sheep or other animal recently killed. Tease the section gently in a drop of salt solution; cover, and examine. The nucleus, nucleolus, and general protoplasm of the nerve cells can be readily seen. The processes of the cells are usually broken off.

If such a preparation be teased in a drop of magenta instead of salt solution, the cells are readily stained, and the axial cylinders of the white nerve fibres may often be found torn out of the medullary sheath and stained.

The membranous capsule, with its nuclei around each cell, may be demonstrated by freezing a fresh Gasserian ganglion, and staining the sections with magenta.

156. Examine (H.) multipolar nerve cells from the hardened spinal cord prepared thus:—Cut the fresh spinal cord of a calf into pieces about a quarter of an inch in length. Place them for a month in one per cent potass. bichrom. solution. Remove a thin slice of the gray matter of the anterior horn with scissors, tease with needles, stain with carmine, and mount in glycerine. Or freeze the fresh cord of the calf; make transverse sections. Place them in $\frac{1}{5}$ per cent osmic acid for twenty-four hours; wash in water, and examine in potassium acetate. Or place them for a day in iodised serum, and then examine.

Each cell has a nucleus and a nucleolus and several processes. With a power of about 1000 diam. the

nerve fibrils may be seen in the processes, especially at the junction of these with the cell, into the granular protoplasm of which they may be traced. If the cells and their processes have been very successfully isolated, it may be seen, as Deiters pointed out, that each cell has one process—the "axial cylinder process"—which undergoes no division, and at some distance from the cell becomes enclosed in the medullary sheath, and thus becomes a white fibre. All the other processes remain *gray;* they repeatedly divide, and form a reticulum in the gray matter of the cord.

The nerve cells of the brain will be examined with that organ.

157. Examine a preparation of the pear-shaped cells of the ganglia of the frog's heart with a power of about 800 diam. Such a preparation may be made as follows (Beale): —Carminise the auricular septum of the frog's heart. Macerate for some days in glycerine (1 oz.), and glacial acetic acid (5 drops); isolate the ganglia by teasing with needles under a dissecting microscope. Preserve in glycerine.

As pointed out by Beale each cell has two processes: a straight fibre resembling in position the stalk of a pear, and a spiral fibre coiled round it for some distance.

PERIPHERAL NERVE TERMINATIONS.

The methods for demonstrating these in the various sense organs will be conveniently studied with these organs themselves.

158. **Plexus of Fibrils.**—This has been already seen in the cornea stained with gold, § 154.

A fine plexus of nerve fibrils may also be beautifully shown in the tail of the tadpole of the green tree frog (*Hyla arborea*), prepared as recommended by Klein. Place the tail of a tadpole in which the posterior extremities are just appearing in ½ per cent solution of chloride of gold for from thirty to forty minutes; wash in distilled water, and leave it in the water exposed to diffuse daylight for twenty-four hours or so. Then strip off the epidermis from one side with a pair of forceps, and mount in glycerine with the inner surface of the epidermis uppermost. The removal of the epidermis is facilitated by immersion in absolute alcohol for fifteen minutes or so. If this preparation be successful, a plexus of nerve fibrils will be found close to the epidermis.

159.—**Pacini's Corpuscles.**—These may be obtained by making a V. S. of the pad of the cat's foot, hardened in chromic acid. Stain with carmine or logwood, and mount in dammar. They may, however, be found with far greater readiness in the mesentery of the cat, where they are evident to the unaided eye as small, clear, oval, bead-like bodies. Examine a corpuscle removed with scissors from the cat's mesentery, and placed in a drop of glycerine.

(L.) The general appearance: nerve fibre entering at one extremity, concentric layers of tissue covering it.

(H.) The axial cylinder of the nerve fibre in the centre of the corpuscle. The medullary sheath disappears as the nerve enters. The axial cylinder is surrounded by a layer of finely granular substance, outside which there are a number of membranes that form a series of concentric tunics. The fresh specimen may be fairly well preserved in glycerine, but they are more perfectly preserved when hardened in chloride of gold or chromic acid.

Each tunic of the capsule of the corpuscle consists of an outer and an inner layer of epithelial scales, with white and elastic fibres surrounded by an albuminous fluid interposed (Key and Retzius). The fibres may be demonstrated by teasing the corpuscle with needles after maceration for a few days in $\frac{1}{6}$ per cent chromic acid (Schaefer, *Quart. Mic. Journ.*, xv. 139).

160. **Nerve Terminations in Striped Muscle.**—These may be most readily found in the muscles of the thigh or back of the lizard (*Lacerta viridis* or *Lacerta agilis*), and in the recti muscles of the eyeball of a rabbit or other animal.

Examine (H.) a preparation made as follows:—With scissors make a thin longitudinal section of a rectus oculi of a rabbit just killed; place it in very dilute acetic acid (two drops of pyroligneous acid in half an ounce of water); and, after a minute or two, gently dissociate the fibres with needles. On carefully looking along the fibres, profile and superficial views of the nerve "end organs" may be obtained. The nerve fibre penetrates the sarcolemma, loses its medullary substance, and appears to end in a plate of nucleated granular protoplasm that forms an eminence

under the sarcolemma (Doyère's eminence); but it is exceedingly doubtful that this plate is the real termination of the nerve, for, after staining with gold, Gerlach has found a plexus of fine varicose fibrils—like nerve fibrils—throughout the muscular substance. (*Sitzungsberichte der Phys. Med. Soc.*; Erlangen, 1873.) Kühne's article on this subject in Stricker's *Histology*, vol. i. p. 202, may be advantageously consulted by the advanced student.

BLOOD-VESSELS.

161. **Structure of Blood-Vessels** (H.)—Blood-vessels may be conveniently prepared from the pia mater; that of a sheep does perfectly. With scissors remove a slice about a quarter of an inch in depth from the surface of a cerebral convolution. Lay it upon a slide, with the pia mater touching the glass. Fix one side of the slice with forceps, and with the *back* of a scalpel *gently* scrape away the brain matter. With a camel-hair brush and water, "pencil" away the remainder. Tease with needles; cover, and examine in water.

a. Capillaries.—Observe the nuclei in the capillaries. They appear to be placed in a homogeneous membrane, but in a silvered preparation it will be seen that the membrane is mapped out by silver lines into polygonal areas, each one enclosing a nucleus. These are, therefore, the nuclei of epithelial squames, with which the whole vascular system is lined. Whether or not a very delicate homogeneous membrane lies outside the epithelium of the capillary is doubtful.

If the capillary be traced up to a large vessel, the first obvious change consists in the addition here and there of a non-striped muscular fibre coiled round the vessel; then the other tissues found in the larger vessels make their appearance.

b. Small arteries and veins.—In small arteries and veins three coats may in general be recognised. The *inner* coat consists of an elastic membrane lined by a single layer of squamous epithelium. The *middle* coat is composed of

non-striped muscular fibres mostly circular in their arrangements, with elastic and white fibrous tissue superadded in vessels of considerable size. In very large vessels, such as the aorta, there is a great preponderance of elastic elements in the form of elastic membranes and fibres. The *outer* coat is made up of elastic and white fibrous tissue. In arteries and veins the same structural elements are generally present, the veins having only thinner walls than the arteries.

a. Search for vessels that appear about an inch in breadth when seen with the power we are at present employing (300 diam.) In the *outer* coat the wavy fibrous tissue will be readily recognised. Inside the outer tunic an appearance of rounded cells will be seen, provided the vessel under examination be an artery; if not, shift the preparation and search for a vessel with this characteristic. This appearance of rounded cells is due to the muscular fibres seen through their long axes as they curl round the vessel. The nucleus appears as a bright refracting spot, if the part of the fibre seen through happen to contain it. With careful focussing the margins of these rounded bodies at the periphery are seen stretching across the vessel as the outlines of the muscular fibres. In the smallest vessels there is only a single layer of muscular fibres, but in those of larger size two and even three layers may be found. The elastic membrane will be more readily seen when the outer and middle coats have been rendered transparent by acetic acid.

b. Add acetic acid. The tunica adventitia swells up, becomes transparent, and the nuclei of its connective tissue corpuscles appear. The muscular fibres become very transparent, and show their more or less staff-shaped nuclei crossing the vessel. With deeper focussing other nuclei may be seen, usually of an oval shape, with their long axes generally parallel with that of the vessel. These belong to the epithelial lining.

The elastic membrane is composed of a transparent highly refractile elastic substance. It may be of uniform extension or fenestrated. It is generally marked by ir-

regular lines that often look like a network of elastic fibres. The vessel under observation has a distinct double contour. The sharp inner line is due to the elastic membrane, and the long somewhat irregular lines seen here and there running along the vessel, generally result from the foldings of this membrane on account of the collapsed state of the vessel. Possibly at the end of a vessel the elastic membrane may be found isolated from the other tissues.

162. Examine (H.) a preparation of the elastic membrane isolated as follows:—Remove the basilar or other artery from the base of the brain, *e.g.*, of a sheep. Take a short piece of the vessel, lay it open, add a drop of acetic acid, tease with needles, and mount in glycerine.

163. Examine (H.) a preparation of blood-vessels of pia mater stained with carmine. It brings out the nuclei of the muscular fibres.

164. Examine (H.) a T. S. blood-vessel of medium size stained with carmine. The muscular fibres, the elastic membrane, and the nuclei of the epithelial lining will be seen. Such a section as this will be found in the preparation of spinal cord yet to be obtained (§ 245), and also in the V. S. tongue (§ 185).

Transverse sections of blood-vessels in their fresh condition may be readily made with the freezing microtome.

164A. Examine capillaries and larger vessels with epithelium silvered according to the method described in § 329. Such a preparation is preserved in glycerine.

Numerous preparations of blood-vessels for preservation will be found in the sections of organs yet to be made.

CIRCULATION OF THE BLOOD.

165. The circulation may be studied in the tail of the fish and tadpole; in the web, mesentery, tongue, and lung of the frog; and amongst warm-blooded animals in the wing of the bat and the omentum of the guinea-pig. The last requires to be kept carefully warmed on a special hot stage devised by Stricker and Sanderson (see *Quarterly Microscop. Journ.* Oct. 1870).

166. **The Frog's Web.**—Inject one, two, or three minims—according to size of frog—of a watery solution of curara, each minim of which contains 0·032 milligramme of the poison. This dose is small, and will take from ten to twenty minutes to produce paralysis. It is well, however, not to give a larger dose, otherwise the vasomotor nerves will be paralysed.

When paralysis has supervened, tie a soft woollen thread gently round the longest toe, close to its tip, and another round the toe next to, and on one or the other side of it. Lay the frog on its back, and stretch the web gently over a triangular window in a piece of cardboard (A, Fig. 44) by drawing the threads attached to the toes gently through the slits *a* and *a'*. The web must be kept wet by the repeated addition of water.

(L.) Artery, vein, capillaries, and pigment cells. The velocity of the stream in the artery is greater than in the vein. This results from the calibre of the artery being less than that of the vein, and from the greater pressure of the blood in the former.

(H.) (Tube of microscope shortened.) Examine the manner in which the coloured and colourless corpuscles move along the capillaries—the former with comparative rapidity, owing to their polished surfaces; the latter tardily, owing to an apparent tendency to adhesion between the wall of the vessel and the corpuscle. In the arteries and veins it may often be seen that the coloured corpuscles move principally in the centre of the stream, while the colourless corpuscles may often be found adhering to the wall of the vessel, or moving tardily along it. The peripheral part of the stream is often devoid of coloured corpuscles (the "lymph space" of older observers). The smaller velocity of the peripheral, as compared with the central part of the stream, is owing to the friction between the vessel and the blood, and the adhesion here and there of the white corpuscles.

167. **The Frog's Mesentery.**—The student is not permitted to do the following experiment, but it may be shown to him by his teacher thus:—The curarised frog is

first stunned by a blow on the head, so that no pain can possibly be produced, and then a vertical incision is made on the *left* side of the abdomen; a vein immediately below the axilla must be avoided, and also the stomach and duodenum. The small intestine is gently drawn out with forceps, and fixed with small pins to a crescent of cork close to a window (B, Fig. 44) in the cardboard. If the ex-

FIG. 44.—Frog-plate of cardboard. The slits *c* and *c'* are only used when the frog is not curarised and requires to be secured in a bag.

amination is to extend over a considerable time, a thick cover-glass should be fixed with sealing-wax to the glass on the cork, leaving, however, the outer half of the latter exposed. The mesentery is spread out on the glass; a small and very thin cover-glass is placed over it, and the membrane is kept moist with aqueous humour. The circulation in the mesentery is extremely beautiful; for the vessels are only covered by a layer of very transparent epithelial scales.

168. **Tail of the Tadpole.**—The circulation may be readily studied in the tadpole's tail. It is only necessary to lay the animal on a slide in a drop of water under a cover-glass. The tadpole is apt to move, however, and therefore it is advantageous to give curara by putting the animal in a watch-glassful of water containing two or three minims of the curara solution (§ 166). It is removed to a slide and covered as before when the palsy is complete. The phenomena of the circulation are similar to those already described in the frog's web, but there is this important difference—the *diapedesis* or emigration of the white blood corpuscles through the walls of the capillaries and small veins, so rarely seen in the frog's web or mesentery unless inflammation be induced, is almost always to be seen in the tadpole's tail under ordinary circumstances: but if a

number of tadpoles be kept for some days in unchanged water, a remarkable tendency to diapedesis manifests itself; the white corpuscles can be found in all stages of emigration. The diapedesis is, however, a very slow process; a corpuscle may take from one to three hours to pass through the vascular wall, thin though it be.

When a small drop of ammonia is placed on the tail, a great emigration of white corpuscles ensues after a time. Red as well as white corpuscles often pass out. The emigration of the former probably results from pressure, for they have no contractile power.

Study the method of injecting blood-vessels (§§ 333-343).

LYMPHATICS.

169. **Stomata of Lymph Sac** (H.) — Examine a silvered preparation of the *septum cysternæ lymphaticæ magnæ* of the frog prepared thus:—

Open the abdomen of the animal just killed, and remove the viscera. The great lymph sac is placed on either side of the spinal column, immediately behind the stomach. Wash away the blood with distilled water, pour a half per cent silver nitrate solution over it, and allow to remain for three or four minutes; wash thoroughly in distilled water, and expose to the light either in distilled water or in glycerine until a brownish colour appear. Excise a portion of the sac and mount it in glycerine.

The silvered outlines of the transparent epithelial plates that form the wall of the septum will be readily recognised. Alter the focus, and observe that there are two layers of these, the cells of the one—the peritoneal—being more or less elongated, those of the other—the layer belonging to the sac—being more rounded in form. In both, the outlines of the cells are somewhat sinuous. The stomata, or apertures leading from the peritoneal into the lymph sac are bounded by a special layer of very finely granular polyhedral cells, that are readily stained throughout by the silver. These cells in diseased states often proliferate, hence they have been designated "*germinating*." (*Klein.*)

Clusters of cells of this germinal serous epithelium may often be found on the mediastinal pleura of the dog, and

on the peritoneal surface of the centrum tendineum of the guinea-pig and rabbit, in the chronic peritonitis induced by tubercle-inoculation. (*Klein.*)

Stomata essentially similar to these may be demonstrated by the above process in other situations, such as the pleura and the surface of the centrum tendineum. Their existence was first made known by Recklinghausen in the latter situation.

170. **Lymphatics of Diaphragm.**—Examine a preparation made as follows :—

Kill a rabbit—a young one is to be preferred—and remove the anterior wall of the chest. Ligature the inferior vena cava near its cardiac end ; separate the pleuræ from the diaphragm, and remove the heart and lungs. Remove the investing serous epithelium by pencilling the upper surface of the diaphragm with a camel-hair brush dipped in the serum found in the chest. Wash the pencilled surface with distilled water ; pour over it a half per cent nitrate of silver solution ; allow to remain for three or four minutes, and then wash thoroughly with distilled water. The diaphragm with its osseous and cartilaginous attachments is then cut out and placed in a vessel with distilled water, until the silvered surface becomes of a brownish colour. The centrum tendineum may then be cut into pieces and mounted in glycerine. The lymphatics near the under surface of the diaphragm may be demonstrated in a similar manner.

(L.) The lymphatics form an anastomosing system of irregular channels of various sizes. The silvered outlines of their epithelial wall are evident. Alternate dilatations and constrictions are often seen on the smaller vessels. The appearance resembles a series of Florence flasks set one above another. There are no valves at the swellings, such as are found in large lymphatics.

(H.) In the smallest lymphatics the epithelial outlines are more sinuous than in the larger vessels, and in the latter the outlines of the cells are more or less parallel with the long axis of the vessel. In favourable specimens, the smallest lymphatics may be seen opening into connective tissue spaces, as described by Recklinghausen. This mode of origin may be beautifully seen in the superficial lymphatics of the intestinal villi (§ 193).

The smallest lymphatics consist entirely of a simple

layer of squamous epithelium. The large vessels, *e.g.* the thoracic duct, have a structure similar to veins.

171. Study the puncture method of injecting lymphatics described in § 344.

Klein's memoir on *The Anatomy of the Lymphatic System*, part i., London, 1873, may be advantageously consulted by the advanced student.

BLOOD GLANDS.

172. **Lymph Gland.**—The methods of preparing lymphatic glands in the fresh condition, and after injection with silver nitrate, have already been described (§§ 110, 111).

Examine the section of lymphatic gland already prepared (§ 110).

(L.) The fibrous capsule sends into the interior numerous trabeculæ that are membranous in the cortex, and cord-like in the medulla. These trabeculæ may be seen cut longitudinally and transversely. Contiguous with the capsule and the trabeculæ, there is everywhere a somewhat clear space, the lymph sinus. From the capsule and trabeculæ a loose adenoid tissue extends across the sinus. The spaces amidst the tissue elements constitute the lymph path continuous with the afferent lymphatics through the cortex, and with the efferent lymphatics through the hilus of the gland. The lymph sinus everywhere encloses a ramifying mass of "follicular tissue," consisting of a somewhat condensed adenoid tissue closely packed with lymph corpuscles.

(H.) The fibres of the trabeculæ. The loose adenoid tissue of the lymph sinus, with probably here and there a lymph corpuscle not shaken out of the spaces (§ 110). The follicular tissue, with numerous lymph corpuscles. The corpuscles are identical with the smaller white blood corpuscles. They appear to be produced in the follicular tissue, and to pass from thence into the lymph stream.

Examine also (L. and H.) a section of silvered lymphatic gland prepared as stated in § 111.

173. **Spleen.**—*Methods.*—*a.* The spleen may be prepared by hardening in chromic acid and alcohol as described in § 11. Sections may be readily made with the hand, or

in the microtome imbedded in paraffin. The sections are mounted in Farrants' solution or in glycerine. They may be unstained or stained with logwood. This dye is especially useful for mapping out the splenic corpuscles.

b. For the study of the splenic pulp, it is advantageous to place small portions of perfectly fresh spleen in one per cent potassium bichromate solution for five or six days, and then to make sections in the freezing microtome. It is difficult to get good sections of the spleen by freezing when it has not been previously hardened by other methods, but the difficulty is entirely overcome by impregnating the tissue with a thick solution of gum previous to freezing (§ 309). The blood corpuscles may be removed from the adenoid tissue of the pulp by gently shaking the sections with salt solution in a test-tube, as recommended for lymphatic gland (§ 110).

c. The cell elements of the spleen may be examined in the fresh condition in aqueous humour.

174. *Structure.*—Examine, without the microscope, the cut surface of the fresh spleen of ox. The tough fibrous capsule is evident. Scrape away the pulp, and observe the branching cord-like trabeculæ continuous with the capsule, and running from one surface to the other of the organ. Look at a cut surface that has not been scraped, and endeavour to see the splenic corpuscles—minute things like millet seeds, or like very small grains of *unboiled* sago. The cut ends of the trabeculæ must not be mistaken for them. Endeavour to pick out a splenic corpuscle with the point of a scalpel. They are generally attached to the tunica adventitia of the arteries. It is sometimes possible to pull out a small artery, and see the corpuscles adhering to it like small beads. The structure of the spleen is somewhat analogous to that of a lymph gland. The capsule and trabeculæ are similar. The splenic corpuscles apparently correspond to the follicular tissue of the latter, while the splenic pulp may be compared to the lymph sinus. In the spleen, however, *blood-vessels* open into the spaces amidst the adenoid tissue of the pulp, to which they appear to be related in a manner similar to that of the

lymphatics to the sinus of the lymph gland; the arteries pass into capillaries, some of which penetrate the splenic corpuscles (W. Sanders). They all eventually open into the spaces in the pulp, from which the venous radicles are derived (W. Müller). There are lymphatics in the spleen around the blood-vessels, and in the trabeculæ; their mode of origin, however, has not been fully investigated.

175. Section of chromic acid spleen of cat stained with logwood. Mount in Farrants' solution or in glycerine.

(L.) Capsule. Trabeculæ. Splenic corpuscles much larger here than they are in the human spleen. The pulp.

(H.) The trabeculæ, chiefly consisting of elastic fibres; non-striped muscle is also present in them and in the capsule. The splenic corpuscles, containing great numbers of lymph corpuscles. The envelope consists of a delicate condensed adenoid tissue. The pulp, consisting of adenoid tissue, with numerous blood-corpuscles in its spaces. Lymph corpuscles are probably produced in the splenic corpuscles, and find their way into the pulp, and from thence by the veins.

176. If the pulp of the fresh spleen be examined in aqueous humour, clear colloid masses containing coloured blood-corpuscles, variously transformed, may be found. These are supposed to be corpuscles breaking up. Similar bodies may be found in the red marrow of bone (§ 129).

177. Examine (L.) a preparation of injected spleen.

178. **Thyroid Gland.**—(L. and H.) Examine sections of human thyroid gland injected and uninjected.

The uninjected gland is best hardened in Müller's fluid for a month, and then in methylated spirit for a fortnight. Preserve in Farrants' solution, or in glycerine, or stain with carmine or logwood, and mount in dammar. The injected gland may be hardened in $\frac{1}{4}$ per cent chromic acid for a fortnight, and then cut by the freezing method, stained with logwood or carmine, and mounted in dammar.

The gland consists of sacs imbedded in fibrous tissue. The sacs are lined by a somewhat cubical epithelium. They contain lymph corpuscles. They are not penetrated by capillaries, as is the case with the splenic corpuscles. In the human subject they are often found enlarged, and filled with a colloid material.

179. **Suprarenal Body** (L. and H.)—Examine V. S. suprarenal body prepared as follows :—Harden in Müller's fluid for a month, and then in methylated spirit for a fortnight. Mount the sections in glycerine or dammar. Groups of nucleated brownish cells are seen with (H.) They are chiefly placed in the outer part of the organ, arranged in rows, or in irregular clusters, in spaces amidst the fibrous tissue.

LUNG.

A section of the trachea has already been examined (§ 115).

180. **Methods.**—*a.* The lung may be extremely well prepared by hardening in chromic acid, and then in alcohol, as directed in § 6.

b. The outlines of the epithelial cells in the alveoli may be silvered as follows :—Allow the lungs to collapse by opening the chest of an animal just killed. Inject a half per cent solution of silver nitrate into a bronchus, and fully distend the lung. Make sections at once in the freezing microtome, using distilled water instead of the gum. Wash the sections in distilled water, and expose them to the light in it. Preserve in Farrants' solution, or in glycerine. Some sections may be good, but the staining is apt to fail.

c. Sections of lung, either fresh or hardened in chromic acid and spirit, are best made with the freezing microtome. The spirit must of course be previously removed by immersing the piece of lung in water for twelve hours or so. Good sections may, however, also be made without a machine, by imbedding the lung in gum, as described in § 301, *b*. The gum is easily removed from the sections by placing them in water.

d. The sections may be examined unstained or stained with logwood. If unstained, they are mounted in Farrants' solution or glycerine ; if stained, they may be preserved in the same fluids, or in dammar (§ 152).

e. The blood-vessels of the lung may be injected, *e.g.* with carmine and gelatine (§ 343). The lung is then hardened in alcohol, as described in § 337, and sections made as above mentioned.

f. The ganglia of the lung may be examined in sections of the lung, hardened in chromic acid in the usual way. They are found

close to the pulmonary vessels and bronchi. The ganglia may also be isolated by cutting out a portion of the wall of a bronchus of a lung prepared as above, and, with the aid of a dissecting microscope, the small ganglia lying outside the cartilages may easily be isolated. (*W. Stirling.*)

181. **Structure.**—An unstained section of the lung of cat across a minute bronchus, made as above described. Mount in Farrants' solution, or in glycerine, and examine.

(L.) Notice the general appearance of the section;—the T. S. bronchus with two or three plates of cartilage cut across;—the larger vessels, the pulmonary artery having a thicker wall than the vein;—the air vesicles somewhat collapsed.

(H.) *The air vesicles.*—The somewhat indefinite fibrillated tissue with numerous elastic fibres, of which the alveolar walls principally consist, will be easily recognised, and also the rounded nuclei of the simple layer of squamous epithelium, with which the alveolus is lined. Capillaries may be found cut across here and there between the alveoli.

The bronchus.—Internally, ciliated epithelium; and externally, plates of hyaline cartilage imbedded in a fibrous membrane. The bronchial glands lie chiefly between the two, but often the sacs of the glands pass between the cartilaginous plates, and occupy a position external to them. Circular non-striped muscular fibres, difficult to recognise *as such*, lie near to the epithelium. Around the glands there is either fibrous or adenoid tissue. The latter is abundantly found in the bronchial mucous membrane, either diffuse or in the form of cords, which, according to Klein, have a longitudinal direction. The cut ends of the elastic fibres that run longitudinally along the bronchi close to the epithelium may also be seen. They are most readily detected in a section stained with logwood. With L. they are seen as bundles causing ridges on the mucous membrane.

The sections of the pulmonary arterioles and veinlets may also be examined.

The internal ganglia of the lung may also be found in this preparation. As Remak pointed out, they are placed on the nerves that accompany the bronchi. The cells are large and multipolar. (*W. Stirling.*)

182. It is advantageous to stain a section of cat's lung with logwood for comparison with the foregoing.

It is also advantageous to have a section of the lung of a child, hardened as described in § 6, cut in the freezing microtome, and stained with logwood. Mount in Farrants' solution. The important point of difference between the cat's lung and this, is the small amount of interalveolar fibrous tissue in the normal human lung. The logwood brings out the nuclei of the alveolar epithelium clearly.

183. Examine (L.) a section of lung with its blood-vessels injected with gelatine and carmine and mounted in dammar (§ 180 *e*); also (H.) a section of silvered lung showing the outlines of the alveolar epithelia.

Regarding the lymphatics of the lung, Klein's memoir, *Anatomy of the Lymphatic System*, Part ii., London, 1875, may be advantageously consulted by the advanced student.

ALIMENTARY CANAL.

TONGUE.

184. **Method.**—The tongue, when cut into small pieces, may be hardened in chromic acid and then in alcohol, as mentioned in § 7. Sections are best made in the freezing microtome, but they may also be made from tongue imbedded in paraffin. The sections may be preserved unstained in Farrants' solution or glycerine, or they may be stained with logwood and preserved in the same fluids or in dammar.

185. **Structure.**—V. S. unstained chromic acid tongue, made as above. Mount in Farrants' solution or glycerine, and examine.

(L.) *The papillæ.*—Unlike the filiform papillæ of the human tongue, those that correspond to them in the cat are short thick stumps. *The muscular fibres* are seen divided longitudinally and transversely. The fasciculi of the latter are well seen, also the general arrangement of the fibres—some passing transversely, others vertically, and others longitudinally.

(H.) *Stratified squamous epithelium.*—The individual cells and their nuclei may be clearly seen in the lower and middle layers; but near the free surface, where it is raised into papillæ, the scales are so compressed that in profile they are not unlike fibres. The cells are jagged in the deeper layers, but this appearance is not so well marked here as in the skin.

186. Examine (L.) V. S. human tongue, showing the fungiform and filiform papillæ; a V. S. of the same, showing a circumvallate papilla; a V. S. injected tongue, showing the capillaries passing into the fibrous tissue at the base of each papilla.

187. **Taste Bulbs.**—Examine (L. and H.) V. S. gustatory disc of rabbit's tongue hardened in absolute alcohol. The sections are stained with logwood, and mounted in glycerine or dammar. The gustatory disc (*papilla foliata*) is found on either side of the dorsum of the tongue near its root.

Each taste bulb consists of a single layer of investing ordinary epithelial cells surrounding a cluster of modified epithelial cells, at the free end of which there is a short and fine hair-like process, and at the other extremity delicate protoplasmic processes that are probably connected with nerve filaments. The bunch of hair-like processes projects into an opening (*gustatory pore*) at the exposed extremity of the taste bulb. In the human tongue the taste bulbs chiefly occur on the outer aspect of the central projection of the circumvallate papillæ.

Engelmann's article in Stricker's *Histology*, vol. iii. p. 1, may be consulted by the advanced student for other methods of preparation.

STOMACH.

188. *Methods.—a.* The stomach may be well hardened in chromic acid and alcohol as described in § 7. Sections may be made in the freezing microtome, or by imbedding in paraffin. The latter is, however, not nearly so good, for the paraffin is very apt to enter the mouths of the glands. The sections unstained are best mounted in Farrants' solution or in glycerine.

b. The cells of the peptic glands are remarkably well seen in a stomach hardened in osmic acid. A portion of the mucous membrane is separated from the muscular coat of the stomach of a cat just killed. The mucus is washed away with a stream of salt solution. One or two pieces about an eighth of an inch square are placed in ¼ per cent osmic acid for twenty-four hours. It may then be readily cut in the freezing microtome, and the sections mounted in glycerine or Farrants' solution. The nuclei of the cells may be well brought into view by *slight* staining with logwood.

c. Sections of the fresh stomach may be readily made in the freezing microtome, and stained with magenta or a watery solution of anilin blue.

189. **Structure.**—V. S. chromic acid stomach of cat; mount in glycerine.

(L.) The relations of the mucous, submucous, and muscular coats.

(H.) *The mucous coat.*—The *peptic follicles* cut vertically. The columnar epithelium at their open ends, and the epithelial cells of irregular shape throughout the greater part of the follicle. The outer or "parietal," and the inner or "chief," varieties of these may be recognised, but these will be better seen in the osmic acid stomach.

Bring into view the *muscularis mucosa* outside the closed ends of the follicles. It consists of two layers of non-striped muscular fibres, bundles from both of which may be traced upwards between the follicles, and may in very thin sections be traced nearly to the open ends of the glands, where, according to Klein, they form a transverse network between the glands.

The submucous coat consists of areolar tissue; blood-vessels may be found here and there.

The muscular coat.—The fasciculi of non-striped fibres can be seen cut longitudinally and transversely.

190. Examine (H.) a V. S. mucous membrane of cat's stomach hardened in osmic acid as above described. If a peptic follicle can be traced throughout its entire length, it will be found that near and at the orifice the epithelium is columnar, with the nuclei placed near the attached ends of the cells. In about the half of the follicle nearest its closed extremity two sorts of cells may be seen. A large, somewhat spheroidal, nucleated cell here and there placed externally close to the basement membrane, and within this, and

occupying the intervals above and below them, smaller cells of irregular shape. The former have long been termed "peptic" cells. By Heidenhain they have been named "parietal," while the others have been termed by him the "chief" cells, from his supposing that *they* are especially concerned in the secretion of gastric juice. About the middle of the follicle the "parietal" cells form an almost continuous layer.

In a V. S. of stomach, near its pyloric orifice, glands, lined throughout by columnar epithelium, may be seen. These are commonly termed mucous glands. They occur, though in relatively small numbers, throughout the remainder of the stomach.

THE INTESTINE.

191. **Methods.**—*a.* The intestine may be hardened in a solution of chromic acid and potassium bichromate, followed by alcohol, as described in § 7. Sections of the small intestine are best made in the freezing microtome, for the villi are apt to be spoiled by paraffin or any similar imbedding agent. The sections unstained are mounted in Farrants' solution or in glycerine, or they may be stained with logwood or carmine, and mounted in the same fluids or in dammar.

b. A method devised by Klein gives good results. Place portions of the intestine in a mixture of two parts of $\frac{1}{6}$ per cent solution of chromic acid, and one part of methylated alcohol. Change the fluid two or three times, and at the end of seven days transfer to dilute spirit (methylated spirit 1, water 2 parts). After two or three days place them in methylated spirit, and when they are sufficiently hard make sections. Place the sections for a few minutes in 1 per cent sodium bicarbonate solution, and then stain them with logwood.

c. Auerbach's plexus between the two layers of the muscular coat may be thus prepared. (*Klein.*) Take the small intestine of a rabbit just killed, and, after washing out its contents with a stream of salt solution, inflate it with air. With broad-pointed forceps strip off the longitudinal layer of muscular fibres in as membranous a form as possible. Place the membranes thus detached in $\frac{1}{2}$ per cent chloride of gold solution for twenty-five minutes; wash in distilled water, and afterwards treat as stated in § 330.

d. The openings of the chalice cells may be rendered very distinct by the silver process already described in § 96.

e. The simple layer of epithelial squames that forms a basement membrane under the columnar epithelium as described by Debove (*Archives de Physiologie*, 1874, p. 19) may be shown without much difficulty thus :—Open the small intestine of a rabbit or cat just killed. Isolate a small portion and wash the mucous membrane with distilled water. Pour $\frac{1}{4}$ per cent silver nitrate solution over the interior, and leave it till it becomes opalescent. Wash in distilled water. Then pencil away the epithelium, and silver the subjacent tissue in the usual way (§ 328). If the villi are then snipped off and mounted in glycerine, the silver lines of a squamous epithelial membrane, somewhat like that of a serous membrane, may be seen.

f. The same process as that just described also serves to show very beautifully the superficial lacteals of the villi and their communications with the spaces in the reticulum of adenoid tissue.

192. **Structure of Small Intestine,** T. S. or V. S.—Small intestine of cat prepared as stated in § 191 *a*. Mount in Farrants' solution or in glycerine. (L.) The mucous coat showing the villi, follicles of Lieberkühn, and muscularis mucosa. The submucous coat. The muscular coat with its two layers of fibres.

(H.) The *columnar epithelium* of the villi with their striated border, and with a chalice cell here and there.

The *follicles of Lieberkühn*, lined throughout by columnar epithelium, the nuclei of which are placed near to the attached ends of the cells. In very thin sections the epithelium may be found detached from the basement membrane. (The membrane consists of simple epithelial squames (Watney), but this can only be seen in silvered specimens.)

The *muscularis mucosa* forming here as in the stomach two layers. Endeavour to trace bundles of fibres from it up into the lateral part of the villus, where they run nearly to its tip.

The *adenoid tissue;* the only connective tissue found in the villi, and also largely found between the follicles of Lieberkühn.

There is nothing remarkable about the submucous and muscular coats.

193. *Lacteals.*—Examine (H.) villi silvered after removal of the columnar epithelium (§ 191, *f*). The clear lines parallel with the villus are the superficial lacteals which

may be seen communicating with the spaces in the adenoid tissue.

194. *Blood-vessels.*—Examine (L.) T. S. small intestine with blood-vessels of villi injected.

195. *Brunner's glands.* — Examine (L.) T. S. duodenum stained with carmine, showing Brunner's glands. They are compound saccular glands.

196. *Peyer's glands.*—*a.* Examine (L.) T. S. or V. S. small intestine, showing Peyer's sacs. These structurally resemble the splenic corpuscles.

b. (L.) A similar section with the capillaries in Peyer's sacs injected.

c. (H.) A similar section showing the lymph corpuscles within the sac.

The solitary glands found here and there in the small and large intestine structurally resemble a single sac of a Peyer's patch.

197. **Structure of Large Intestine.**—Unstained, T.S. or V. S. large intestine of cat prepared as directed in § 191, *a.* Mount in Farrants' solution or in glycerine. (L.) The follicles of Lieberkühn in the mucosa. The submucous and muscular coats.

(H.) Lieberkühn's follicles similar to those of the small intestine.

198. Examine (H.) T. S. follicles of Lieberkühn in order to be able to recognise tubular glands such as those when divided transversely.

LIVER.

199. **Methods.**—*a.* The liver may be very successfully hardened in Müller's fluid, and then in rectified spirit, as directed in § 10. A one per cent solution of potassium bichromate for a fortnight or so, followed by rectified spirit, also gives very fair results.

Sections of the hardened liver, imbedded in paraffin, may readily be made. The sections should be examined unstained and also stained with logwood, a dye peculiarly suitable for the liver and kidney. In either case they should be mounted in Farrants' solution, or in glycerine.

b. The blood-vessels and bile ducts may be injected as directed in §§ 343, 346.

200. **Structure.**—*Cells* (H.)—*a.* Scrape the cut surface of the fresh liver of rabbit or cat; diffuse the scraping in a drop of salt solution; cover and examine. The cells have one—rarely two—nuclei; the protoplasm is distinctly granular, and there is no envelope.

b. Prepare in the same manner the cells of the liver of the ox. Particles of fat are usually to be seen in these.

201. Thin section of liver of cat hardened in Müller's fluid and spirit (§ 199, *a*). The section should be made across one of the larger bile ducts. Stain with logwood, by placing the section on a slide in a few drops of strong logwood solution for two or three minutes. Wash in water; and mount in Farrants' solution, or in glycerine.

(L.) *The lobules.*—Observe the *network* of the gland cells in each. If the section be across the long axis of a lobule, branching columns of cells may be seen radiating from the centre. A network of capillaries occupies the spaces between the cells. The T. S. of one of the larger bile ducts may perhaps be seen, with a section of the hepatic artery and portal vein near it. If there be a T. S. of the bile duct in the preparation, it is convenient to place it exactly in the centre of the field, otherwise it will not be readily found with H.

(H.) The *bile duct* lined by a layer of columnar epithelium. There is nothing remarkable about the sections of the hepatic artery and portal vein.

The capsule of Glisson.—A fine fibrous tissue that surrounds all the vessels and runs between the lobules. The whole liver is enclosed in a thin fibrous capsule. The tissue now seen is a prolongation of the capsule around the vessels that enter at the portal fissure. The capsule also sends fine trabeculæ between the lobules from many points of the surface. Look for these near the margin of the section. (They are most readily seen, however, when the cells have been pencilled away.)

The columns of cells are easily seen, and in the intervals between the columns, collapsed capillaries with blood corpuscles.

Mount an unstained section of liver in Farrants' solution, and compare it with the preceding.

202. *Blood-vessels* (L.)—Section of liver of cat, injected from the portal vein with Prussian blue and gelatine, and hardened in rectified spirit. Mount in dammar, and examine. The radicles of the hepatic vein lie in the centre, the terminations of the portal vein between the lobules and capillaries connect the two venous systems.

203. *Bile ducts* (H.)—Examine a section of rabbit's liver having the bile ducts injected with soluble Prussian blue (§ 346), and the venous system injected with carmine and gelatine, mounted in dammar. The larger (interlobular) bile ducts are filled with injection, around which the columnar epithelium may be seen. Lateral branches may be readily traced into the lobules. The columnar epithelium becomes shorter and shorter, and soon disappears. The branches ramify, and soon open into a network of fine ducts—the *bile capillaries* between the individual hepatic cells. These capillaries appear to be mere rounded spaces between the cells. If, after having seen this injection, the student carefully look with H. at the uninjected preparation already made (§ 200), he may see here and there fine pores between the cells—the empty bile capillaries—much smaller than the spaces that contain the blood capillaries.

204. *Glands of the Bile Ducts.*—The large bile ducts have numerous mucous glands which may be seen in T. S. of the duct, most readily after they have been filled with injection from the common bile duct. Examine (L.) a preparation showing these.

SALIVARY GLANDS AND PANCREAS.

205. **Methods.**—*a.* These organs if cut into sufficiently small pieces may be well hardened in a saturated solution of picric acid, as recommended by Ranvier. About forty-eight hours are required. Sections may then be readily made with the freezing microtome. They may be mounted unstained in Farrants' solution, or in glycerine; or they may be stained with picro-carmine, and mounted in the same fluids.

b. The glands may also be hardened in alcohol, and sections made with the hand. The sections may be stained with Beale's carmine fluid (§ 318, *a*), diluted four or five times with water.

c. Osmic acid is also a good hardening agent. Small portions of the glands are hardened for two days in $\frac{1}{6}$ per cent solution. They may then be cut by freezing, or may be farther hardened in alcohol and cut by the hand. This method of hardening is especially suitable when it is desired to dissociate the gland elements with needles. The sections are mounted in Farrants' solution, or in potassium acetate solution.

206. **Structure.**—The salivary glands and pancreas have an essentially similar structure. They are all racemose glands; the ducts lined by columnar epithelium, and the acini containing polyhedral epithelial cells, with channels for the secreted fluid occurring here and there between them. The secreting cells of the submaxillary gland differ from those of the parotid and pancreas in containing mucin.

Examine (L.) a section of submaxillary gland, prepared stated in § 205, *a*. The acini.

(H.) The cells of the acini and those of the ducts.

KIDNEY.

207. **Methods.** — (*a.*) The epithelium of the fresh kidney may be examined by merely scraping the surface of a Malpighian pyramid cut vertically, and examining the scraping in salt solution.

b. Sections of the fresh kidney may be made with a Valentin's knife (Fig. 56), but far better with the freezing microtome. Sections of the unhardened kidney can scarcely be preserved.

c. The best ordinary methods for preparing the kidney are precisely the same as in the case of the liver. Therefore all that is stated in § 199, *a* applies equally to both.

d. Small pieces of kidney may also be hardened in a solution of chromic acid. The kidney of the rabbit may be very successfully hardened in 1 per cent solution, as directed in § 9.

e. The renal tubules may be isolated (Ludwig) by making thin vertical sections of the Malpighian pyramids of the fresh kidney, and putting them in a flask containing one part of hydrochloric acid to four parts of rectified spirit. A cork, with a long glass tube passing through it, is fitted to the flask, and it is boiled over a sandbath for two or three

hours, and then the mixture is replaced by distilled water, in which they are left for a day. The tubules are then isolated by shaking a slice in a test tube, with a little water.

f. The epithelium lining the Malpighian capsules is best demonstrated in the kidney of the child at birth. Cut it into small pieces. Place them in 1 per cent potassium bichromate solution for a week, then in ¼ per cent chromic acid for a day, and make sections in the freezing microtome.

g. The blood-vessels may be readily injected with gelatine and carmine, etc., as described in § 342.

208. **Structure**.—Examine V. S. Malpighian pyramid without the aid of the microscope; the whitish compact medullary substance near the apex of the pyramid; the softer reddish-brown cortex at the base. The longitudinal striæ in the medullary substance are due to the straight tubules. The pyramids of Ferrein—bundles of the straight tubules—are easily seen where the medullary passes into the cortical substance. The red lines between these pyramids are due to the vasa recta. In the cortex it is just possible to see fine granulations on the cut surface, produced by the glomeruli. Tear off the fibrous capsule, and in so doing notice the fine filaments that appear to be torn across; these are capillaries that pass between the cortex and the capsule.

209. V. S. Malpighian pyramid of cat's kidney hardened in Müller's fluid, as described in § 9, cut in the freezing microtome, and stained with logwood. Mount in Farrants' solution or in glycerine.

(L.) Trace the tubules from the apex of the Malpighian pyramid into the cortex. The tubules are straight in the medulla, and form bundles—the pyramids of Ferrein—towards its outer part. These pass nearly to the outer part of the cortex. In the cortex the straight tubules give off lateral branches that become the convoluted tubules, and finally end in the capsules enclosing the glomeruli. These lie amidst the convoluted tubules, and can be readily recognised.

(H.) The glomerulus—or Malpighian body—a cluster of capillaries enclosed in a capsule formed by expansion of the basement membrane of a convoluted tubule lined by

squamous epithelium. Profile views of the nuclei of the epithelial cells will probably be obtained. The squamous epithelium of the capsule is reflected over the capillaries of the glomerulus, but it is scarcely possible to see this, save in the young child. Examine the epithelium of the convoluted and that of the straight tubules.

210. Mount an unstained V. S. Malpighian pyramid, and compare it with the above. Especially look at the appearance of the unstained epithelium of the tubules.

211. Examine (H.) V. S. Malpighian pyramid of rabbit's kidney hardened in chromic acid (§ 207, *d*). The columnar epithelium of the larger straight tubules can be remarkably well seen near the apex of the pyramid. The epithelium of the convoluted tubules is not so good as in the Müller's fluid kidney. The divisions of the straight tubules may be seen; and the small looped tubules of Henle may often be recognised.

212. (H.) Scrape the cut surface of the cortex of fresh kidney of sheep to get isolated glomeruli, epithelium, and basement membranes. Examine in salt solution, then add acetic acid and notice its effect on the glomerulus. It renders the capillary walls extremely transparent, and reveals their nuclei.

213. (H.) T. S. Malpighian pyramid of Müller's fluid kidney near its apex. Examine and preserve in Farrants' solution or in glycerine. (The section is best made from a frozen kidney, but may also be made with a Valentin's knife.) Study the T. S. tubules, and the matrix of connective tissue between them. The connective tissue is evident in the medullary part of the kidney, but it is very scanty in the cortex.

214. V. S. Malpighian pyramid of injected kidney. Mount in dammar, and examine.

(L. and H.) See the interlobular arteries in the cortex; the glomerulus, each with its afferent vessel derived from the interlobular artery and the efferent vessel breaking up into a second set of capillaries around the convoluted tubules. See also the vasa recta between the pyramids of Ferrein.

URINARY DEPOSITS.

The various forms of these are studied in the chemical division of the class of Practical Physiology. It is convenient, however, to study here the methods of mounting them, in order that the student may prepare specimens similar to those which he will afterwards be shown.

215. **Collection and Examination.**—Allow the urine to deposit in a conical glass, and remove, with the aid of a long pipette, a portion of the sediment for microscopical examination, or remove the supernatant fluid with a syphon, in order that the deposit may not be diffused through the fluid by the disturbing influence of the pipette. For the ordinary examination of the deposit it is generally convenient to place a drop of the urine on a slide with a shallow cell. The best cell for this purpose consists of a rounded shallow cavity ground in the centre of the surface of the slide. A cell made of a thin ring of dammar varnish, allowed to dry, also answers the purpose. The cell prevents the diffusion of the deposit under the weight of the cover-glass. A magnifying power of 250 is most suitable. Those who employ Hartnack's microscope, with the No. 7 objective and No. 3 ocular, should shorten the tube fully, as the power is a little too high with the tube elongated—that is to say, the lens requires, in the latter case, to come so near the cover-glass that the whole depth of the cell is not visible.

216. **Mounting.**—The deposit should be washed at least twice with the fluid in which it is to be mounted, or in rectified spirit in the case of such deposits as uric acid, etc., when they are to be mounted in dammar or Canada balsam. For this purpose allow the urine to deposit. Remove the supernatant fluid with a syphon, or decant it; pour on the preservative fluid; allow deposit to form; remove fluid with a syphon or a pipette, again pour on preservative fluid, and keep the mixture until wanted. When deposits are to be mounted in fluid media they should always be placed in a shallow dammar or balsam cell, made by painting a ring of fluid dammar, or Canada balsam, on a slide. With the aid of a

pipette, place a particle of the deposit in such a cell, add a drop of the preservative fluid, and seal up.

217. **Urates.**—Mount in weak spirit or glycerine solution (see § 221).

218. **Cystine.**—Mount in dilute acetic acid (ordinary pyroligneous acid 1 part, water 20 parts).

219. **Triple Phosphate.**—Mount in strong ammonia 1 part, water 6 parts.

220. **Calcic Phosphate** is usually amorphous, but sometimes occurs in rosettes of crystals. These may be readily obtained by simply adding to urine a small piece of calcium chloride. The precipitate is allowed to form in a conical glass, and may be mounted and preserved in the mother liquor.

221. **Uric Acid and Oxalate of Lime.**—Mount in equal parts of *glycerine and camphor water*. (Camphor water is thus prepared: wrap 3 grammes of camphor in a piece of muslin, place it in a bottle containing 1500CC of water, shake repeatedly. The fluid is ready for use in three or four days). Or they may be mounted in glycerine jelly as follows. Wash the deposit in weak spirit. Heat the glycerine jelly in a water bath until it becomes fluid. Place a drop on a warm slide, add the urinary deposit, and after a few seconds apply a warm cover-glass. When the jelly is cold, scrape it away from the edge of the slide, and seal up with zinc cement, to prevent the hygroscopic jelly from attracting moisture from the air. As the jelly answers admirably, the use of Canada balsam or dammar is unnecessary; and this is fortunate, for the use of these media in this connection is attended with considerable difficulty.

222. **Casts and Epithelium.**—*Pus* and *mucus* may be preserved in naphtha and creosote fluid prepared as follows:—(*Beale*) A, creosote, 35CC; B, wood naphtha, 28CC; C, precipitated chalk, 130 grammes; D, water, 600CC. Mix thoroughly A and B and C in a mortar; very gradually add D, keeping the mixture in motion with the pestle all the while. Place the whole, together with a few pieces of camphor, in a lightly covered vessel for two

or three weeks, occasionally shaking or stirring the mixture, then filter, and preserve in corked or stoppered bottles. (*Beale.*)

SKIN.

223. **Methods.**—*a.* Very good preparations may be made of the skin hardened in the mixture of chromic acid and spirit, as described in § 16. The sections may be advantageously stained with picro-carmine. They are best mounted—stained or unstained—in Farrants' solution or in glycerine. Stained sections may, however, also be mounted in dammar.

b. The method of digestion may be applied to the skin with advantage (*W. Stirling*) for the purpose of studying the arrangements of the muscular and elastic fibres. Mix 1CC pure hydrochloric acid with 500CC water, and add 1 gramme pepsine.* After keeping the mixture at 38° C. for three hours, shake it thoroughly. Stretch a piece of skin of man or dog, as fresh as possible, over the mouth of a glass dialysing jar, and tie it firmly round the jar to keep it stretched. Digest the skin in the above fluid at 38° C. for a period varying from two to eight hours, according to the size and age of the skin. Young skin digests more quickly than that which is old. It is advantageous to use only about 100CC of the digestive fluid at one time, and to change the fluid every second hour if the piece of skin be large, in order to remove the peptones, and thereby facilitate the digestive process. After partial digestion, place the skin in water for twenty-four hours. In this it becomes swollen and transparent. It can then be hardened in the ordinary fluids, and stained with logwood or carmine. By the above process the white fibrous tissue swells up and becomes extremely transparent, thus permitting of a clear view of the other tissues.

c. The outlines of the cells of the *rete mucosum* may be well shown by treatment with osmic acid, as described in § 87B.

d. The epidermic cells may be rendered prone to separate by maceration in potassium bichromate solution, as described in § 87C.

e. Staining with gold and osmic acid is serviceable for the demonstration of the structure of the tactile corpuscles and their connection with nerves. (See Thin, *Journ. of Anat. and Phys.*, vol. viii. 30.)

224. **Structure of the Skin.**—Examine the V. S. human skin already prepared (§ 87).

(L.) In the epidermis observe the rete mucosum below,

* The *pepsina porci* of the British Pharmacopœia may be conveniently employed.

the horny layer above. The lower stratum of the latter is clear and almost homogeneous in appearance.

In the cutis vera observe the papillæ, the areolar tissue, sudoriferous ducts; probably the coils of the ducts and fat cells will be found in the deeper stratum.

(H.) The *epidermic cells* have already been examined (§ 87), but it will be well to complete the subject by looking at the clear layer at the junction of the horny layer with the rete mucosum,—sometimes spoken of as the layer of Langerhans. It consists of epidermic cells so condensed that it is scarcely possible to see their outlines, unless the section be stained with such a dye as picro-carmine.

The *papillæ* consist of a compact fibrous tissue. Deeper in the cutis the tissue becomes distinctly areolar.

The *sweat glands* are simple tubules that will probably be found cut in various directions. In the cutis there is a basement membrane lined by the gland cells. In the cuticle the sweat canal is an irregular somewhat twisted channel amidst the epidermic cells.

A *touch corpuscle* may be found in a papilla here and there, somewhat oval in shape, and having a fibrous appearance. A nerve fibre may, perhaps, be seen in connection with it. (The nerve fibre passes to the summit, and then ends within the corpuscle in a soft core that is enclosed in a tunic of fibrous tissue. This, however, can only be seen when the corpuscle has been rendered very transparent, *e.g.*, by acetic acid.)

Pacinian corpuscles may possibly be found in the *lower* part of the cutis (seeing that the skin under examination is from the palmar surface of the finger). The structure of these has been already considered (§ 159).

225. Examine (L.) V. S. skin of human heel. The sudoriferous glands may be very distinctly seen in this situation.

226. Examine (600 diam.) a tactile corpuscle in an extremely thin V. S. of skin from palmar surface of point of human finger.

227. **Human Hair.** (H.)—Pluck out a hair of the head—with its bulb, if possible; place a drop of dammar

upon it, cover and examine. In a hair three parts may usually be distinguished :—1. The *cuticle*, consisting of a layer of epidermic scales arranged in an imbricated fashion ; 2. The *cortex*, composed of a substance which often appears almost homogeneous, with a few longitudinal lines and granules scattered through it, but which, on bruising a hair that has been boiled in sulphuric acid, is found to consist of epidermic scales elongated and resembling fibres ; 3. The *pith*, consisting of irregular cavities amidst the epidermic fibres. These cavities are often absent. The pigment of the hair is either in the form of masses of granules scattered through the cortex, or is diffused throughout the epidermic matter.

228. **Hair of Sheep.** (H.)—Mount in dammar. Observe the strongly marked borders of the cuticular cells, readily seen in profile.

229. **Hair of Rabbit** (H.)—Mount in dammar. Observe the single or double row of cavities in the pith, and the relatively small amount of fibrous cortex.

230. **The Hair Follicle.**—The hair in its follicle is enclosed in envelopes derived from the dermis and epidermis. From without inwards, the envelopes are—

I. *Dermic Coverings.*

1. A layer of white fibrous tissue with its bundles arranged *longitudinally.*

2. A fibrous layer of somewhat indeterminate character, the connective tissue corpuscles and the indistinct fibres around them being arranged *transversely.*

3. A hyaline layer of doubtful structure, resembling a basement membrane.

II. *Epidermic Coverings.*

4. The *outer root sheath*, consisting of a mass of cells irregularly disposed, continuous with the rete mucosum of the adjoining skin.

5. The *inner root sheath*, composed of two layers :—

a. Henle's layer (*fenestrated* layer), made up of a

stratum of flat non-nucleated cells with irregular spaces between them.

b. Huxley's layer, composed of a stratum of flat nucleated cells without fenestræ.

The structure of layers *a* and *b* can only be clearly seen where they have been isolated by dissection.

6. The *cuticle of the root sheath*, formed of a layer of imbricated cells in contact with the cuticle of the hair.

The inner root sheath and its cuticle seldom pass higher than the openings of the sebaceous glands.

231. Examine the following preparations:—*a.* (H.) T. S. hair follicle from scalp, preserved in glycerine or dammar. Layers 1, 2, 4, 5 will be readily seen. 3 and 6 will probably be difficult to recognise.

b. V. S. hair follicle of scalp.

(L.) The follicle, usually somewhat oblique in position, with the sebaceous gland or glands opening into the upper part.

(H.) The fibrous coverings of the follicle and the outer root sheath may be readily seen, but it will probably be difficult to see the others clearly.

c. (L.) V. S. skin of dog digested (§ 223, *b*) and preserved in glycerine. Examine the erector muscle of the hair follicle, composed of non-striped fibres passing from the lower part of the hair follicle to end in elastic fibres close to the surface of the *cutis vera*, at a little distance from the follicle, but on that side of it towards which the hair slopes. It is rare to see the whole length of the muscle in a single preparation.

EYE.

232. **Cornea.**—*Methods.*—*a.* The cornea may be readily hardened in chromic acid, as directed in § 13. This method serves very well for the demonstration of the epithelium, the elastic lamina, and the fibrous tissue. It may be readily cut if imbedded in paraffin, and the sections unstained or stained with logwood, mounted in Farrants' solution or in glycerine.

b. The nerves and connective corpuscles of the cornea may be stained with gold chloride, as described in § 330, *a*.

c. The cell spaces amidst the corneal fibres may be readily demonstated as follows : *—The epithelium is scraped away from the anterior surface of the cornea of a living frog anæsthetised by chloroform, or stunned to prevent all pain, and one or two drops of a 2 per cent solution of nitrate of silver are introduced within the conjunctiva. At the end of fifteen minutes the cornea is excised, placed in glycerine, and exposed to diffuse daylight till it is of a brown colour. The cornea may be split into layers with scissors and forceps. This will be found advantageous, for the transparency of the cornea is greatly diminished by silvering, and the outer layers are rendered useless by the process. The preparations are mounted in glycerine. Such preparations may also be made from the cornea of the rabbit, anæsthetised with ether.

233. **Structure.**—V. S. chromic acid cornea of cat, prepared as stated in § 232, *a*. Mount in Farrants' solution, and examine.

(L.) Anterior epithelium.—Fibrous tissue. Elastic lamina with posterior epithelium. Unless the section be made by freezing, the posterior epithelium is very apt to be torn off.

(H.) Several layers of the anterior epithelium, the lower cells resting immediately upon the fibrous tissue without the intervention of a thin elastic lamina—Bowman's membrane —as in the case of the human cornea.

The single layer of epithelium placed posteriorly on the membrane of Descemet—a thick elastic lamina.

The fibrous tissue with the nuclei of connective tissue corpuscles (corneal corpuscles).

234. Examine (H.) a cornea silvered by the method described in § 232, *c*. The fibrous tissue is darkened by the silver, and the connective tissue spaces are readily seen forming an anastomosing system of channels ("lymph canalicular system") in which the corneal corpuscles are placed, and through which the lymph from the marginal capillaries can readily permeate.

235. Examine (600 diam.) the nerves and corneal corpuscles stained with gold by the method described in § 330, *b*. Plexuses of nerve fibrils lie between the layers of fibres, and may be exposed by stripping off the fibrous layers from say the *anterior* surface of the cornea. Nerve fibres

* The student is not permitted to do this.

are seen dividing into fibrils, and these forming a network without—even when examined with very high powers—any apparent connection with surrounding tissue. The nerve fibrils have almost always a beaded appearance.

236. **Crystalline Lens** (H.)—Fibres of lens of cod fish boiled for ten minutes in dilute sulphuric acid (1 per cent). Dissociate with needles and preserve in Farrants' solution. The serrations of the fibres are very distinctly seen. The lens of the ox, cat, or rabbit may be prepared in the same manner, but the serrations are not nearly so well marked.

237. Examine *a.* (L.) T. S. lens hardened in absolute alcohol. The laminæ of fibres are readily seen.

b. (H.) T. S. fibres of lens hardened in chromic acid and spirit (§ 13), showing their hexagonal shape.

238. **Hexagonal Pigment Cells** (H.)—With the point of a scalpel scrape off some of the pigmentary layer inside the choroid of an eye hardened in chromic acid and spirit, § 13, and place the scraping in a drop of Farrants' solution. Cover, and examine. The hexagonal cells, each with a clear nucleus, will be readily seen.

239. **Retina.**—*Methods.—a.* The retina may be well prepared for section in the chromic acid and spirit fluid, as mentioned in § 13. The sections may be unstained or stained with dilute carmine (§ 318, *a*) and preserved in Farrants' solution or in glycerine.

b. The retina may also be well hardened by osmic acid. It is placed in a $\frac{1}{4}$ per cent solution for thirty-six hours, and then washed in distilled water for twenty-four hours or so. Sections are preserved in Farrants' solution, or in a saturated solution of potassium acetate. Osmic acid is the best agent for showing the general form and connections of the rods and cones. These may be isolated by teasing sections made by freezing, as described below, or more simply by means of scissors.

c. The gold method is very useful (*W. Stirling*) for facilitating the cleavage of the outer segments of the rods transversely into their plates, as described by Max Schultze. The posterior half of the eyeball of a frog is placed for an

hour or so in a $\frac{1}{2}$ per cent solution of gold chloride, and after thorough washing in water, and exposure to diffuse light for two days in water slightly acidulated with acetic acid, it will be found that on teasing out a small piece of the retina the outer segments of the rods readily tend to cleave transversely.

d. To make good sections of the retina has hitherto been a matter of the greatest difficulty, for any imbedding agent that may be used is so apt to spoil the bacillary layer. The freezing microtome has, however, overcome the difficulty completely, and it is easy to obtain any number of perfect sections with its aid.

240. **Structure** (H.)—V. S. retina of cat prepared as stated in § 239, *a*. Place the ends of the section towards the ends of the slide, add a drop of Farrants' solution. Cover, and examine.

The general scheme of the structure of the retina is this (*Schultze*):—Delicate nervous elements are supported by a framework of connective tissue. The rods and cones are the terminations of the fibres of the optic nerve with which their connection is probably the following. A nerve fibril passes from the inner segment of each rod, and has one or two nuclear swellings upon its course inwards. From the inner segment of each cone there passes a bundle of fibrils, which has also at least one nuclear swelling upon its course inwards. Both the rod and cone fibrils join multipolar nerve cells at the inner part of the retina, which in turn are connected with the fibres of the optic nerve. The actual continuity of the rod and cone fibrils with the multipolar nerve cells has not, however, been traced as yet.

The connective tissue elements principally consist of a membrane (*inner limiting membrane*) on the inner aspect of the retina, and another (*outer limiting membrane*) inside the rods and cones, with trabeculæ (*fibres of Müller*) passing between the two. Nine or even ten layers may be distinguished in the retina which we have now to examine in detail in the preparation.

1. *Inner limiting* membrane, with the fibres of Müller extending from it outwards.

2. *Layer of nerve fibres* consists of the axial cylinders of the optic nerve fibres.

3. *Layer of nerve cells* is composed of multipolar cells, the central process of each being an undivided axial cylinder process continuous with an optic nerve fibre, and the external processes, several in number, dividing again and again, and thus giving rise to smaller bundles of fibrils that pass outwards into the next layer, through which, however, they have not as yet been traced.

4. *Inner granular layer* ("inner molecular layer"). With this magnifying power (300) it appears to be nothing but minute granules, but when very highly magnified a *clear matrix* containing fine granules may be seen. The fibres of Müller pass through it, and probably so do the nerve fibrils.

5. *Inner nuclear layer* contains distinct nuclei, each with a nucleolus. A few of them belong to the fibres of Müller; the majority, however, belong to the nerve fibrils passing outwards. (The nerve fibrils cannot be distinctly seen without a much higher power.)

6. *Outer granular layer* is much thinner, but is in other respects similar to the inner granular layer; its limits are much more easily seen in a retina stained with carmine, for then the inner and outer nuclear layers are stained, while the granular layers are not.

7. *Outer nuclear layer* contains many nuclei, the majority of which belong to the *rod* nerve fibrils, one nucleus upon each fibril placed at various parts of their course. The fibrils, however, can only be seen with a very high power, and best in the osmic acid retina. Transverse striation of these nuclei first pointed out by Henle is very evident; the general substance of the nucleus is highly refractile, and is crossed by one, sometimes two stripes of a less refractile substance. There is no nucleolus. It is much more difficult to find the single nucleus with nucleolus upon the cone fibre placed just within the outer limiting membrane. It has no stripes, and is exactly like the nuclei of the inner nuclear layer. It is best seen in the osmic acid retina of the frog.

8. *Outer limiting membrane.*
9. *Rods and cones* (Jacob's membrane, or the bacillary layer).
10. *Pigmentary layer.*

} Cannot be clearly seen in this preparation.

The pigmentary layer consists of the hexagonal pigment cells already examined (§ 238), hitherto commonly regarded as the inner layer of the choroid, but included by Max Schultze as one of the layers of the retina, because of delicate connective tissue fibrils, apparently connected with the cells, passing inwards between the outer segments of the rods and cones.

240A. Examine the following preparations :—*a.* V. S. osmic acid retina of frog prepared as directed in § 239, *b*, and cut by freezing. A power of 300 diam. will do, but 600 is much to be preferred. The rods being far larger than in the cat's retina are easily seen, each consisting of an outer and an inner segment. The former will probably be much blackened by the osmic acid. It is normally as clear as glass. The inner segment is seen perforating the extremely thin *outer limiting membrane.* A cone is usually seen with difficulty. Its outer segment is shorter than that of the rod.

b. (H.) Rods of frog's retina hardened in gold (§ 239, *c*). Observe the outer segments of the rods cleaving transversely into discs. The outer segments of a cone cleave in like manner. According to Schultze the inner segments of rods and cones are the true nerve terminations, while the outer segments consist of columns of plates that play the part of a reflecting apparatus.

c. (L.) V. S. optic nerve entering eyeball, tinged with carmine, showing the prominence (*papilla*) of the nerve, its central blood-vessels, and the fibres of the lamina cribrosa of the sclerotic, through the spaces between which the nerve fibres enter the eyeball.

d. (L.) V. S. from before backwards, of ciliary muscle, iris, sclerotic, cornea, choroid and ciliary processes. The anterior attachment of the muscle to the cornea is seen, and its fibres are seen to spread out posteriorly on the choroid.

e. (L.) Inner aspect of ciliary processes injected—opaque or transparent.

e. Branched pigment cells of the substance of the choroid. These may be seen in any thin section of the tunics of the eyeball.

COCHLEA.

241. **Methods.**—*a.* The structure of Corti's organ may be investigated in the fresh condition, but, except in very young animals, little can be learned by such a mode of examination regarding the relations of the various soft tissues. For this purpose, Corti's organ must be hardened, its bony surroundings softened, and the cochlea sliced vertically. It is of the greatest importance that the organ be placed in preservative fluid as soon after death as possible. If the human cochlea be prepared, it should be taken not later than twelve hours after death; and in order to preserve it as thoroughly as possible, it is well, immediately after death, to inject rectified spirit into the tympanic cavity with an ordinary subcutaneous syringe thrust through the membrana tympani.

b. Hardening.—Osmic acid and chromic acid are the best hardening agents. The latter is to be preferred when sections are to be made of the surrounding bone. The best chromic fluid is the mixture of chromic acid and spirit (§ 4, Solution 3). This fluid is used in the manner indicated in § 17.

c. Softening the bone.—In the case of fœtal cochleæ, the bone may be softened by chromic acid alone, but for older cochleæ a stronger acid should be added. German authors recommend for this purpose hydrochloric acid diluted with water ten times, but far better results are obtained by using the bone-softening fluid already mentioned. (See § 4, Solution 5.) A large quantity of this fluid is necessary, and in the case of the larger cochleæ, they should be kept in motion by some such means as an ordinary meat-jack. With the aid of this device, even a large cochlea may be softened in a few days.

d. Section.—When sections are to be made of the softened cochlea, the lamina spiralis and organ of Corti require support to maintain them *in situ*. Melted wax and a hot solution of gelatine have both been used for this purpose with some success, but the method of imbedding in gum is decidedly preferable. Remove the cochlea from the softening fluid, and place it in a small cone of bibulous paper, containing a strong solution of gum arabic for four or five hours, then immerse the cone in methylated spirit for forty-eight hours or so, until the gum has become hard and tough; then remove the superfluous gum, and imbed the whole in the ordinary paraffin mixture in a microtome (§ 303), with the modiolus of the cochlea placed at right angles to the axis of the tube of the microtome, so that Corti's organ will be sliced vertically. Or transfer the cochlea from the softening fluid to a solution of gum placed in the well of the freezing microtome, and, after letting it soak in the gum for some hours, freeze and make sections. After either the one or

other mode of section, the slices should be immediately placed in rectified spirit.

e. Staining.—The sections may be stained with logwood, silver, and carmine. The last two yield the best results. Carmine may be successfully used in the following way. Mix equal parts of water, methylated spirit, and Beale's carmine fluid, with all the ammonia retained (see § 318A). (The spirit is added to the mixture in order to prevent the rapid swelling up and solution of the supporting gum.) Transfer the sections from the rectified spirit to the above carmine fluid, and let them remain in this for about twelve hours, until a deep diffuse staining is obtained. Then pour away the carmine and wash the sections very gently with dilute alcohol (rectified spirit 1 part, distilled water 3 parts). Next cover them with an acid washing fluid, consisting of rectified spirit 60 parts, water 39 parts, hydrochloric acid 1 part. After washing in this fluid for six or eight hours, until all diffuse staining is removed, cautiously pour off the fluid and preserve the sections in ordinary rectified spirit until required. Silver staining is useful for the fresh tissue. It reveals the outlines of the epithelium of the cochlea. The process of staining is the ordinary silver method (§ 328).

f. Mounting.—Great care is necessary in mounting the sections, lest important parts be injured. If the sections have not been stained, the gum must be carefully removed or softened by the above, but if glycerine jelly be used for mounting, this precaution is unnecessary. The best preservative media are glycerine, glycerine jelly, and dammar. The last is only resorted to when the sections have been stained. For unstained, and even for stained sections, glycerine and glycerine jelly are good preservative media. In mounting vertical sections of the cochlea, the modiolus should always be placed across the glass slip to facilitate subsequent microscopical study.

These methods give good results, and they were to a large extent devised by my late assistant, Dr. Pritchard, to whom I gave this as the subject for a thesis for the University of Edinburgh.

242. **Structure.**—Examine a series of preparations hardened in chromic acid and unstained or stained with carmine.

a. (L.) V. S. cochlea showing modiolus. Lamina spiralis ossea and membranacea, with the organ of Corti on the latter.

b. (H.) V. S. cochlea showing inner and outer rods of Corti, both planted on the basilar membrane. The head of the inner rod resembles somewhat the head of the ulna. The head of the outer rod is articulated with it. A short process passes outward from the head of each rod, to which the *membrana reticularis* here seen in section is connected. The outer rods are longer than the inner ones.

The *cells of Corti* will be easily seen external to the outer rods. There are three rows in the cat and some other animals, four in man. Each cell has short stiff hair-like processes on its upper extremity projecting through the membrana reticularis. A process passes from the lower end of the cell to be attached to the basilar membrane. Outside Corti's cells the epithelium is columnar, but without hairs. It gradually becomes short and cubical, and so passes gradually into the epithelium lining the outer part of the scala media.

The *membrana basilaris* may be seen stretching from the lamina spiralis ossea to a fan-shaped (in V. S.) expansion — the *ligamentum spirale* of Kölliker (the *cochlear muscle* of Bowman). The tissue of the ligament is peculiar, and it is difficult to say what is precisely its nature.

The *membrana tectoria* may be seen extending from the *limbus laminæ spiralis* over the heads of the rods and the hair cells. In the normal condition it appears to hang free above these, but very often in prepared cochleæ it lies closely upon the hair cells. According to Waldeyer it consists in the unhardened cochlea of a soft pulpy material.

The *membrane of Reissner* may perhaps be found. It consists of a single layer of flattened epithelial cells separating the scala media from the scala vestibuli.

The *spiral ganglion* may be seen in carminised sections —within the bone of the spiral lamina.

c. (H.) A preparation showing a superficial view of Corti's organ ; the heads of the rods resembling a row of pianoforte keys ; the membrana reticularis consisting of plates that have the form of rings and phalanges. The hairs of Corti's cells project through the rings.

SCHNEIDERIAN MEMBRANE.

243. Examine (H.) a preparation of olfactory cells from the nose of the newt, prepared thus :—Harden the head in Müller's fluid for four or five days. Snip out the upper part of the nostril. Scrape the mucous membrane of the detached portion with the point of a scalpel. Dissociate with needles,

and preserve in Farrants' solution. The olfactory cells are long and slender, with a large nucleus about the centre. The ordinary epithelial cells are much broader. In the newt and frog the former have delicate hairs on their peripheral extremities, but these can rarely be seen.

SPINAL CORD AND MEDULLA OBLONGATA.

244. **Methods.**—*a.* The spinal cord and medulla oblongata may be hardened by immersion in rectified spirit followed by a $\frac{1}{4}$ per cent solution of chromic acid (*Lockhart Clarke*) as directed in § 14. Sections are made in a microtome with the cord imbedded in carrot or paraffin. The sections may be stained with carmine or logwood. The former gives good results if the staining be very slowly done. The dilution of Beale's carmine fluid with all the ammonia retained (§ 318, *a*) produces excellent results. Twenty-four hours or more may be required. Carmine may, however, completely fail if the tissue have been over-hardened by chromic acid. In that case the carmine staining may be readily effected after the previous action of palladium chloride, as described in § 319, or the sections may be stained with logwood.

b. The sections, whether stained or unstained, are usually mounted in dammar.

c. A method for isolating the nerve cells of the cord has been already described in § 156.

245. **Structure of the Cord.**—*a.* T. S. spinal cord of cat carminised. Mount in dammar (§ 152).

(L.) The anterior and posterior median fissures. The gray matter brightly stained with carmine. The nerve roots may perhaps be seen; the anterior forming two or three bundles, the posterior only a single bundle. The anterior, lateral, and posterior white columns in each half of the cord. The gray (posterior) and the white (anterior) commissures uniting the two halves of the cord. The central canal in the gray commissure. Leave the canal exactly in the centre of the field, and change the lens.

(H.) The *central canal* lined by a single layer of ciliated

columnar epithelium; the cilia, however, have been destroyed in the hardening process.

The gray matter with its multipolar cells relatively larger in the anterior horn. The nerve fibres in the white columns mostly cut transversely with the axial cylinders stained. The pia mater sending in here and there well-marked processes continuous with a fine connective tissue (neuroglia) lying between the nerve elements. The processes contain an elastic tissue which may be shown by pencilling a section of the hardened cord. In the spinal cord, medulla, and perhaps in other parts at the base of the brain, the neuroglia is a nucleated connective tissue, the matrix of which is fibrillated. In the cerebrum and cerebellum the matrix appears to be a sort of jelly, but it is difficult to define it exactly.

b. Mount an unstained T. S. cord in dammar for comparison with the preceding.

246. Examine the following preparations :—

a. (L.) V. S. white and gray columns of spinal cord carminised.

b. (L.) T. S. spinal cord injected. The gray is far more vascular than the white matter.

246A. **Structure of Medulla Oblongata.**—Examine the following preparations :—

a. (L.) T. S. carminised medulla oblongata through the decussation of the anterior pyramids. Observe the decussation; the enlarged tips of the posterior horns of gray matter, now termed the tubercles of Rolando; the posterior pyramids.

b. (L.) T. S. carminised medulla through the olivary bodies, and the lower part of the fourth ventricle. The following parts may be recognised from the anterior median line outwards in either half of the medulla. The anterior pyramid without any gray matter. Fibres of the hypoglossal nerve passing to the gray matter at the floor of the fourth ventricle. The olivary fasciculus with its ganglion—the *corpus dentatum*—a crumpled sheet of gray matter. Fibres of the vagus nerve passing to the gray matter at the floor of the ventricle. The restiform tract and its gray matter.

The posterior pyramids are now difficult to distinguish from these. They contain gray matter. The floor of the fourth ventricle with its gray matter is evident.

CEREBELLUM AND CEREBRUM.

247. **Methods.**—*a.* The methods of preparation are similar to those recommended for the spinal cord and medulla. The brain, however, cannot bear a solution of chromic acid so strong as that recommended for the cord. The dilute solution of chromic acid and potass. bichrom. (Solution 4, § 4), used as directed in § 14, gives good results. The sections are treated as in the case of the spinal cord.

b. The brain may also be hardened first in rectified spirit, and then in a 2 per cent solution of potass. bichrom.

c. The cells of the cerebrum may be prepared for examination in their fresh condition by a method devised by Mr. Lewis of Wakefield (*Monthly Microscop. Journal*, vol. xvi. p. 105). The pia mater is stripped from a convolution of the human cerebrum as fresh as possible. Thin vertical sections of the gray matter are then made with a broad-bladed and perfectly sharp razor, the surface of which is deluged with spirit. As thin a section as possible is floated from the razor upon a slide, and Müller's fluid is added, to make a pool of fluid above and below the section, so that the tendency of the nervous matter to adhere to the glass during the next process may be diminished. The cover-glass is then applied and cautiously pressed down with the point of a needle applied to its centre, until the nervous matter becomes a thin transparent film. The slide is then placed in a flat dish and covered with methylated spirit. After about forty seconds the slide is removed from the dish, one edge of the cover-glass is steadied with the finger, while the other is slowly raised with the point of a scalpel. The spirit facilitates the removal of the cover-glass, and the nerve film is left floating on the slide, or adhering to the cover-glass. To stain the nerve cells a drop of a 1 per cent solution of aniline black (§ 325) is placed on the film. When the colour is sufficiently deep the slide is lowered under water in a shallow vessel, and washed by gently moving the water with a brush. Previous to mounting, the slide, with the film uncovered, is placed under a bell glass with sulphuric acid, and when perfectly dry a few drops of clove oil, or still better, chloroform, are placed on the film, then a drop of dammar, and then the cover-glass. Staining agents other than the above mentioned may be used.

248. **Structure of Cerebellum.**—V. S. cerebellum of man or of cat, carminised. Mount in dammar.

(L.) The primary and secondary convolutions. The

white matter within and two layers of gray matter outside, the inner of the two being the more intensely coloured, and having a *granular* appearance with this magnifying power. The cells of Purkinje may just be detected lying at the inner part of the outer gray layer.

(H.) *Outer gray layer.*—The cells of Purkinje have a single central process that passes into the inner gray layer. It is often very difficult to see this, but there is no difficulty in seeing one or two processes passing from the outer aspect and dividing again and again, and thus forming a network in the outer gray layer as the branching processes of the cells of the spinal cord do in its gray matter. The nuclei scattered here and there belong to the neuroglia.

In the *inner gray layer* great numbers of nuclei are easily seen. They closely resemble the nuclei of the neuroglia.

248A. **Structure of Cerebrum.**—*a.* V. S. convolutions of carminised human cerebrum. Mount in dammar.

(L.) The convolutions are simple in character—without secondary folds as in the cerebellum. In very favourable specimens a lamination of the gray matter may be seen. This indeed in the unhardened brain is evident to the eye, assisted by a simple lens. There are alternate white and gray layers in the *gray* matter, but little heed need be given to an examination of these with a low power, for one of the layers that has a whitish appearance contains most of the pyramidal cells that are so characteristic of the cerebral ganglion.

(H.) It is really very difficult to succeed in getting a specimen just thin enough, and just sufficiently well stained, to show the five layers described in the *gray* matter by Lockhart Clarke and Meynert. The student may very possibly see little more than the pyramidal cells ; even these will not be sufficiently seen if the section be not perfectly vertical. The pyramidal cells occur about the middle of the gray matter, of which they are the largest cells. Their apices are directed outwards, numerous processes come off from the base, and usually one from the apex. According to Meynert, all the processes ramify excepting one—the

axial cylinder process—connected with the centre of the base of the pyramid.

From without inwards the layers of the gray matter, as seen in very good specimens, are :—

1. A thin layer, consisting almost entirely of neuroglia.

2. A thin layer of minute closely placed nerve cells of irregular shape, with branching processes.

3. A layer three or four times the breadth of either 1 or 2, containing the pyramidal cells, scattered throughout its whole thickness.

4. A thin layer of closely set small granule-like corpuscles of irregular shape.

5. A layer twice as broad as the last, consisting of cells, for the most part of fusiform shape. This layer gradually merges into the central white matter of the convolution.

Fine fasciculi of medullated fibres pass outwards in the gray matter. Other fasciculi of medullated (*Meynert*) fibres run at right angles to these, and leave interstices that are occupied by the nerve cells and the reticulum formed by their branching processes (*Gerlach*).

b. Examine a V. S. cerebral convolutions injected, and observe the greater vascularity of the gray as compared with the white matter.

GENERATIVE ORGANS.

MALE ORGANS.

249. **Testis.**—*Methods.*—Mihalkovics (Ludwig's *Arbeiten*, 1874, p. 19) recommends the following method of hardening the testis for the purpose of section, as preferable to all others :—Inject, with a sharp-pointed syringe, 1 per cent osmic acid at several points through the tunica albuginea of the testis of a cat; place it in rectified spirit for several days, and then in absolute alcohol for a day or two, previous to making sections. Stain with carmine or logwood, and mount in glycerine.

b. Hardening in Müller's fluid, and then in alcohol, is also a fairly good method for studying the cells in the *tubuli seminiferi* in teased preparations.

c. For the isolation of the tubules, Sappey long ago recommended the maceration of small portions of the organ in dilute hydrochloric acid (acid 1, water 2 parts) for one or two days.

d. The lymphatics of the testis may be readily injected by the method described in § 344.

250. *Structure.*—*a.* T. S. testis of cat hardened in osmic acid and alcohol; mount in glycerine.

(L.) Tunica albuginea; mediastinum and septa passing from it to the tunica. Tubuli seminiferi.

(H.) Nucleated cells—the spermatoblasts, and perhaps also spermatozoids, within the tubuli seminiferi. The membrane of the tubules is thick, and, according to Mihalkovics, consists of several layers of flattened cells, that are unaffected, and may, indeed, be demonstrated by a twelve hours' immersion in strong hydrochloric acid.

b. Examine (L.) *a.* V. S. testis of cat, prepared as above, showing tunica albuginea, tubuli seminiferi, tubuli recti, rete testis, and perhaps also the vasa efferentia, passing to the epididymis.

c. Examine (H.) a preparation of spermatozoids of ox or sheep, preserved by drying.

251. **Prostate.**—This may be hardened in a $\frac{1}{4}$ per cent chromic acid, followed by alcohol.

FEMALE ORGANS.

252. **Uterus.**—This may be readily hardened in a $\frac{1}{4}$ per cent chromic acid followed by alcohol (§ 11). T. S. uterus of cat, unstained; mount in glycerine. (L.) Mucous membrane and muscle. (H.) The uterine glands; tubular, and lined by columnar epithelium.

253. **Ovary.**—*Methods.* The ovary may be exceedingly well prepared by hardening in Müller's fluid and alcohol, as directed in § 12A. The sections are best stained with logwood, and may be mounted in glycerine or in dammar.

254. *Structure.*—Examine a section of the ovary prepared as directed above.

(L.) Graafian follicle surrounded by a fibrous stroma.

(H.) The following parts may be recognised from within outwards in a mature Graafian follicle:—1. The *germinal spot;* a nucleolus. 2. The *germinal vesicle;* a nucleus. 3. Granular protoplasm; the *vitellus.* 4. The *zona pellu-*

cida; the envelope of the cell. 5. The *tunica granulosa* covering the *zona.* 6. The space that contains the *liquor folliculi* placed outside the tunica. 7. The *membrana granulosa* lining the ovisac. 8. The *cumulus proligerus* uniting the *tunica* with the *membrana.* 5, 7, and 8 consist of epithelial cells. 9. The *ovisac* composed of a fibro-vascular tissue.

255. Examine (H.) the section of an ovary of a young kitten, showing here and there one of the epithelial cells of the columnar epithelium on the peritoneal aspect of the ovary enlarged. This, as described by Foulis, becomes the nucleated protoplast of the future ovum. It becomes enclosed by connective tissue corpuscles growing from the stroma up around it. These eventually produce the cells of the tunica and membrana granulosa, which for a long time form one mass, but eventually become separated by the appearance of the liquor folliculi. The zona pellucida is an after formation, possibly a sort of excretion derived from the protoplasm of the ovum, or from the cells of the tunica granulosa.

The tubes which have been described in the ovary appear to be nothing more than sulci produced by the unequal outward growth of the ovary (*Foulis*). Young ova may be found growing on the surfaces of the sulci in the same manner as on the free surface of the ovary.

DEVELOPMENT.

256. Examine a series of models of the human embryo, and preparations of the embryo chick. A description of the structure of the latter, and of the details of the methods of preparation, would carry us beyond the limits of these outlines. The advanced student will, however, find a complete account of the subject in the *Elements of Embryology*, by Foster and Balfour. (London: Macmillan, 1874.)

PART IV.

GENERAL CONSIDERATIONS REGARDING HISTOLOGICAL METHODS.

METHODS OF APPLYING RE-AGENTS TO THE TISSUES.

257. **Application of Fluids.**—*Irrigation.* When a microscopic object is to be irrigated, a drop of any desirable fluid is placed on the slide close to the margin of the cover-glass, and if necessary a strip of bibulous paper is dipped in the fluid at the opposite side, to remove that which is to be replaced. For the removal of thick fluids such as glycerine, it is necessary to use a pipette (Fig. 45), which may be readily made by blowing a bulb on a glass tube, and drawing one extremity of the tube to a sufficiently fine point. (Read § 57, *i.*)

258. **Application of Vapours and Gases.**—An example of a simple method for applying a vapour to a microscopic object is given in § 89, where the chloroform vapour is applied to cilia. A drop of the fluid is placed on the floor of a glass cell, and the tissue is placed on the under surface of a cover-glass inverted over it.

For the application of gases, it is necessary to have two tubes in connection with the cavity of a cell: one for the entrance, the other for the exit of the gas. The warm stage, to be presently described (Figs. 48, 49), is so arranged that it may serve as a gas chamber for the attainment of this object. Various gases, such as carbonic acid, oxygen, etc., may be employed.

For the production of carbonic acid an apparatus such as that shown in Fig. 46 is convenient. Dilute hydrochloric acid is placed in a bottle A, and pieces of broken

marble in another bottle C. When the gas is desired, A is elevated upon some support. The gas is passed through a wash bottle (C) containing water, and thence to a T tube (D). The microscopic gas chamber is joined to the tube E; and when the transmission of gas through it is desired, a clamp (G) is placed upon the waste-tube F. When it is

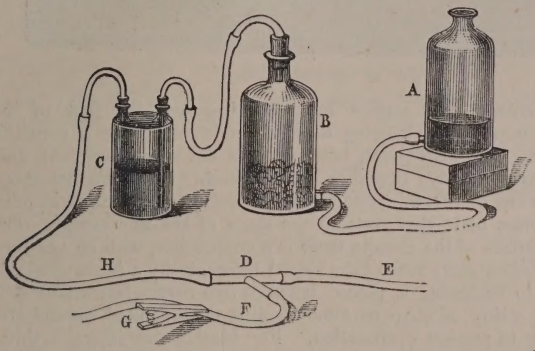

FIG. 46.—Carbonic acid apparatus.

necessary to rapidly arrest the transmission of the gas, a clamp is placed on H. The gas accumulates in B, and drives the acid back into A. A stream of air may be pumped through the gas cell by connecting an elastic pump to F, and placing the clamp G upon H.

FIG. 45. Pipette for removing fluid from microscopic objects.

259. **Application of Heat.** — *Simple hot stage.*—A stage suitable for roughly heating a microscopic object may be made of a plate of tin $9 \times 2\frac{1}{2}$ inches, with an aperture about a quarter of an inch in diameter in the centre (Fig. 47). The plate should be long enough to project some inches on each side of the stage of the microscope. The slide, with the object to be heated, is laid on the plate, the object is focalised, and a spirit lamp is applied to one end of the plate. It is convenient to apply the lamp to the right extremity, and to keep the forefinger of the left hand on the plate

close to the slide, so that the lamp may be removed the moment the temperature becomes unpleasant to the touch.

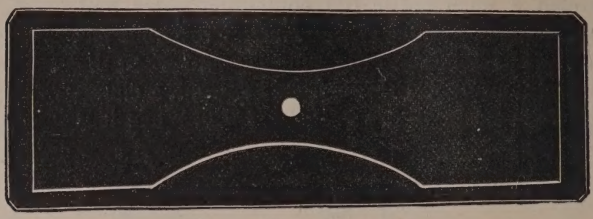

FIG. 47.—Simple hot stage.

260. *Stricker's hot stage.*—Max Schultze was the first to invent an apparatus for keeping a microscopic object at a constant temperature. His instrument, however, was not very convenient, and modifications of it have therefore been devised. Stricker's hot stage (Fig. 48) is one of these. It consists of a rectangular piece of ebonite fixed on a brass plate that rests on the stage of the microscope. On the upper surface of the ebonite there is a copper disc, with an aperture (*c*) leading into a short metal tube closed inferiorly by a piece of glass. The object to be heated is placed between two cover-glasses and laid on the disc, a ring of olive or almond oil is then painted around the cover-glasses to prevent evaporation. For heating the object, a thick copper wire (*w*), coiled at one end, is stuck upon the copper tube (*a*),

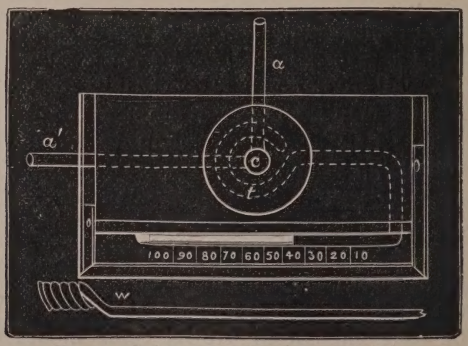

FIG. 48.—Stricker's hot stage and gas chamber.

by the wall of which the heat is conducted to the copper disc. The wire is about six inches long, and is heated by a spirit lamp or gas-burner placed on the table in front of the microscope. The temperature depends on the distance of the point of application of the flame from the stage, and on the proximity of the flame to the wire. The tempera-

ture of the copper disc is indicated by a thermometer (*t*). The temperature which it shows, however, is only approximately that of the object. This method of heating such a stage is convenient, because of the rapidity with which it may be used. When, however, a constant temperature is to be maintained for any considerable length of time, the stage is best heated by a stream of hot water, as suggested by Stricker and Sanderson (Fig. 49). Before passing to it, however, it should be stated that the tubes *a* and *a'* (Fig. 48) are inlet and outlet tubes for the conveyance of gases through the cell. The wall of the tube *a* is used as a heat-conductor merely for convenience.

261. **Stricker and Sanderson's Hot Stage and Gas Chamber** is the best for maintaining a constant temperature. It (Fig. 49) is a hollow flattened brass box through which hot water flows by the tubes *i* and *o*. There is a central cell similar to *c* in Fig. 48, with a thermometer (*t*) coiled round it. Two tubes, *a* and *b*, for gases, communicate with the cell. Recently Dr. Sanderson has adapted to *this stage the wire-heating apparatus* shown in Fig. 48, so that the same stage may be heated by the wire or by hot water—a great convenience.*

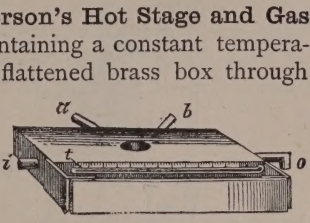

FIG. 49.—Stricker and Sanderson's hot stage.

262. The simplest arrangement for supplying a stream of hot water at a constant temperature is that (Fig. 50) devised by Mr. Schaefer (*Quarterly Microscop. Journ.*, vol. xiv. p. 349). *a* represents a vertical section of the cell in the stage. *e e* is an indiarubber tube for the circulation of water through the stage from the reservoir B. The water is heated by a gas-jet, the size of which is regulated by the expansion of mercury in the apparatus A. When the stage has attained the desired temperature, the mercury A is elevated by the screw S until it touches the metal tube, *f*. The gas can then only issue through the lateral slit in the tube, so that the jet is reduced to a very small size. It readily enlarges, however, the moment the mercury falls.†

These forms of the hot stage are only adapted for keep-

* This form of the stage is made by Hawksley, Oxford Street, London.

† This apparatus is made by Casella, 147 Holborn Bars, London.

ing small objects at a constant temperature. Stricker and Sanderson have described a larger stage, whereby such an object as the omentum of the guinea-pig may be kept

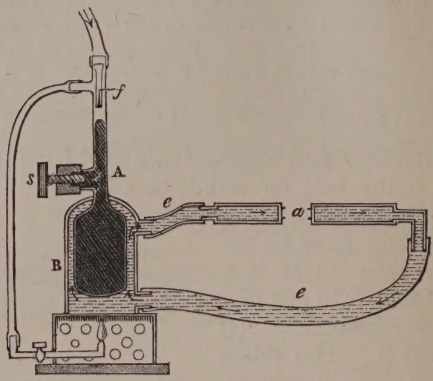

FIG. 50.—Schaefer's apparatus for heating warm stage.

at a constant temperature for the study of the circulation. (See *Quarterly Microscop. Journ.*, Oct. 1870.)

263. **Application of Electricity.**—Various complicated arrangements have been devised for the transmission of electricity through microscopic objects. The following simple arrangements, however, answer the purpose perfectly. Take a slip of glass 1 × 5 inches, and of the thickness of an

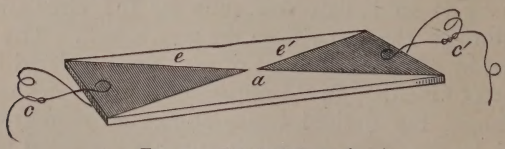

FIG. 51.—Gold-leaf electrodes.

ordinary slide.* Cover one surface with a thin layer of gum-arabic or gold size; press the moist surface firmly down on gold-leaf; allow it to dry; scrape away the gold-leaf, so as to leave two triangles, *e e′* (Fig. 51), with their

* An ordinary slide might be used for these electrodes, but it is convenient to have the slide so long that its ends project beyond the stage.

apices pointing towards each other at the centre of the slide, leaving an interval of about a tenth of an inch between them (*a*) for the reception of the object, which is covered with thin glass in the usual way.

Electricity may be readily transmitted through the electrodes by clamping the slide at each end with an ordinary brass mounting clip (*c c'*), round which thin wire is twisted. If induced electricity be employed, the wires are attached to the secondary coil of Du Bois Reymond's induction apparatus, with a key interposed in the secondary circuit; while in the case of voltaic electricity, the wires are attached to the poles of one or more voltaic cells, with a key introduced into the circuit.

METHODS OF STUDYING LIVING TISSUES.

264. In studying the normal characters of living tissues when removed from the body of an animal, they are either examined in the fluids that bathe them during life—*e.g.*, blood, salivary, mucus, and pus corpuscles are investigated in their own fluids,—or the tissues are studied in other fluids calculated to produce little or no change in their vital properties. Such fluids are termed "neutral" or "indifferent."

265. **Serous Fluids.**—The fluid which bathes most tissues is lymph. *Blood serum*—seeing that it has a composition resembling that of lymph—is sometimes employed for moistening living tissues; but the blood corpuscles that it contains are an inconvenience. *Aqueous humour* is the most available serous fluid when only a small quantity is desired. It can always be readily obtained from the eye of a sheep or ox. It is somewhat difficult to puncture the cornea without losing the aqueous humour. Beer's knife (Fig. 57) is suitable for the purpose.

266. Serous fluids are also employed in the examination of non-living tissues when these are prone to undergo change—*e.g.*, nerve fibrils, red marrow cells, spleen pulp, etc. When they are required in considerable quantity, *amniotic fluid*, *pericardial fluid*, *iodised serum*, or *dilute*

albumin, are employed. These may be preserved for a considerable time by placing them, together with a piece of camphor, in glass vessels, which have been thoroughly cleansed by *boiling* water, to destroy bacterial germs or other causes of putrefaction.

267. *Iodised serum* is thus prepared:—Add 1CC tincture of iodine and one or two drops of carbolic acid to 100CC *fresh* amniotic fluid, and filter.

268. **Dilute Albumin** should only be had recourse to when serous fluids cannot be obtained. It is thus prepared:—Dissolve 2 grammes dried sodium chloride in 250CC water; add 28 grammes egg albumin, 2·3CC tincture of iodine, two or three drops carbolic acid. Mix thoroughly, and filter.

269. **Salt Solution** is a $\frac{3}{4}$ per cent solution of common salt prepared thus:—Heat sodium chloride to redness, cool it over sulphuric acid, and dissolve 7·5 grammes in 1000CC distilled water. This fluid, though often termed *indifferent*, is not in reality so. It is very convenient, but a serous fluid is always to be preferred when *indifference* is really desired.

270. **Moist Chambers.**—Living tissues require to be kept moist. For this purpose they are placed in a chamber, the air of which is kept saturated with moisture.

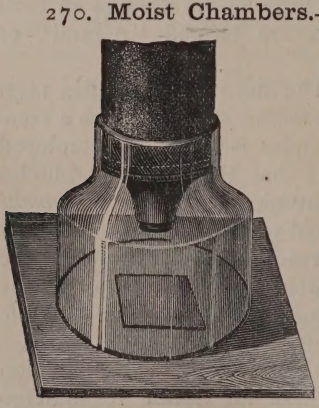

FIG. 52.—Recklinghausen and Schultze's moist chamber.

Schultze's modification of Recklinghausen's moist chamber is a well-known form of this apparatus (Fig. 52). It is a glass cylinder—similar to the lower part of an ordinary lamp-chimney—fixed to a plate of glass. The object is laid under a cover-glass; the air of the chamber is kept moist with strips of wet blotting-paper. The tube of the microscope fits into the cylinder, and evaporation is prevented by a piece of soft leather interposed between the two. In the original chamber devised by Recklinghausen, a piece of thin caoutchouc was tied round a simple glass cylinder

below, and the tube of the microscope above. This chamber is convenient enough for *low*, but *not* for *high* powers; and in any case the air in the chamber tends to become dry. This difficulty, together with that experienced in using a moist chamber with very high powers, such as the $\frac{1}{25}$ objective, has been entirely overcome by Mr. Dallinger and Dr. Drysdale.

271. *Dallinger and Drysdale's Moist Chamber.*—This apparatus (*Monthly Microscop. Journ.*, vol. xi. p. 97), consists of a plate of glass about a tenth of an inch thick, and of a size suitable for the stage of the microscope, room for its movement in all directions being allowed. (A, Fig.

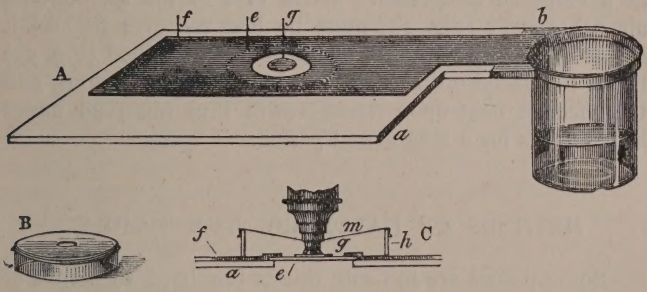

FIG. 53.—Dallinger and Drysdale's moist chamber. A, Glass stage. B, Glass cell with caoutchouc membrane. C, Vertical section of stage and cell, with lens. ½

53). At one side of the glass plate (*a*) there is a rectangular arm, to which a brass plate (*b*) with a ring is fixed. The latter holds an ordinary beaker with water. The glass plate being too thick for the illumination of an object with a powerful achromatic condenser, a piece is cut out of its centre, and thin glass cemented over the aperture (*e* in A and in C). Moisture is continually supplied by a strip of thin calico (*f*) spread over the plate and dipping into the water in the beaker. An aperture must be made in its centre about two or three times the breadth of the cover-glass (*g*) employed. The inventors found an aperture $\frac{11}{16}$ inch for a cover-glass of $\frac{1}{4}$ inch work well. A thin piece of caoutchouc, with an aperture in its centre, is tied over a glass cylinder (B) about $1\frac{1}{2}$ inch in diam. and $\frac{3}{4}$ inch in

height. The cylinder is set upon the cloth, and the lens (*immersion*) is screwed down through the aperture in the elastic membrane, as represented in vertical section in C, where *a* is the thick and *e* the thin glass plate of the moist calico, *g* the cover-glass upon the object, *h* the glass cylinder, and *m* the caoutchouc membrane in contact with the objective *immersed* in a drop of fluid on the cover-glass. Any evaporation from the chamber is fully compensated by the passage of fluid through the cloth by capillary attraction below the margin of the cylinder.

272. When it is necessary to preserve the living tissue at a constant temperature above that of the surrounding air, a hot stage (Fig. 49) must be had recourse to, and it seems likely that the moist chamber last described (Fig. 53) might be placed on a hot stage of suitable dimensions, and the effect of heat upon tissue-growth thus observed under high powers for a lengthened period.

METHODS OF HARDENING THE TISSUES.

Some tissues are too soft, others too hard, to permit of their being cut or dissected; it is therefore necessary to alter their consistence.

273. **Drying and Boiling** were formerly much resorted to, but are now rarely employed. The former may be used for tendon (§ 104), when transverse sections are wanted merely to show the relations of the various systems of fibres; moreover, it is sometimes advantageous to *partially* dry a tissue that has been hardened in alcohol before attempting to cut it. If, however, protoplasm and other soft matters have not been previously hardened, they are generally spoilt by desiccation. Boiling is useful for hardening the fibres of the crystalline lens (§ 236), and also those of muscle, when coarse dissections are to be made of the latter.

274. **Chromic Acid** is much employed as a hardening agent. A dilute solution of it in water may be used alone for hardening the lung, spleen, uterus, muscle, cornea,

spinal cord, and nerve; a dilute solution of chromic acid with potassium bichromate is used for the brain, stomach, and intestine. A solution of chromic acid in dilute alcohol is used for the retina, cochlea, and skin (see pp. 4 and 5). It is not well adapted for the liver, kidney, lymphatic gland, crystalline lens, and vitreous humour.

Chromic acid hardens the tissues in from two days to two months. The tissue should be cut into small pieces and placed in a small quantity of fluid for the first eighteen hours. This should be changed at the end of this period, and a *large* quantity of fluid substituted. The process of hardening by means of chromic acid appears to be analogous to the process of *tanning*. The tissue becomes tough, like leather. The chromic acid is removed from the fluid, and possibly forms some compound in the tissue, hence a large quantity of the chromic solution is necessary. The solution employed is always dilute; the same object cannot be attained by using a strong solution of the acid, for in that case an impervious crust forms around the outer part of the tissue, while the centre remains soft and becomes rotten. When the tissues are sufficiently hard, they are removed from the acid and placed in methylated spirit, if it be necessary to preserve them until sections are made. Very delicate tissues that are apt to spoil in the chromic acid, *e.g.* the embryo, should be placed in methylated spirit ere they are sufficiently hard. The spirit completes the process of induration. Spirit removes much of the yellow colour given to the tissues by the chromic acid.

Chromic acid is a very hygroscopic substance, and therefore, as it is very difficult to keep dry, it is advisable to keep it dissolved. A 1 per cent solution is most convenient, and can be diluted at any time.

275. **Picric or Carbazotic Acid** is a hardening agent introduced by Ranvier. A saturated solution made with cold water is employed. The pieces of tissue must be *small* and the quantity of fluid *large*.

It does not render the tissues so hard as chromic acid, but at the same time its tendency to produce shrivelling is less. It is recommended in § 205 as a hardening agent

for the salivary glands and pancreas. While hardening albuminous tissues it removes calcareous matter; on this account it is useful in the preparation of bone (§ 119). It gives the tissues a deep yellow tinge, which may, however, be removed by immersion in water or in alcohol. In the case of chromic acid the colour is much more difficult to remove.

276. **Müller's Fluid** is thus prepared :—

Potassium bichromate, 2·5 grammes.
Sodium sulphate, 1 gramme.
Distilled water, 100CC.

This fluid is very useful for hardening the liver, kidney, ovary, etc. (pp. 4 and 5). It is sometimes termed Müller's *eye fluid*, on account of its having been originally employed for hardening the retina. For this purpose, however, it is inferior to a mixture of chromic acid and alcohol. After the use of Müller's fluid for a month or so, the hardening is carried still farther by the employment of rectified spirit.

277. **Potassium Bichromate** is the hardening agent in Müller's fluid. This substance may, however, be employed alone. A 1 or 2 per cent solution in water is useful for hardening glands; from two weeks to a month are required. Hardening is carried still farther by immersion in rectified spirit, which also removes much of the colour.

278. **Alcohol** is useful when rapid hardening is required. It acts by removing water and coagulating albumin. It is one of the oldest of indurating agents. It is specially useful for yellow fibro-cartilage (§ 114) and gland tissues. The ordinary rectified spirit (sp. gr. 0·838), and absolute alcohol are the best forms. Methylated spirit, often recommended, is to be avoided. The tissues should be placed first in equal parts of rectified spirit and water for a day; then in rectified spirit for forty-eight hours, and finally in absolute alcohol for some hours, to complete the hardening. The tissues spoil if they are allowed to remain too long in the alcohol, unless they have been previously hardened in chromic, picric, or osmic acids, or potassium bichromate and such like. Alcohol is now rarely employed alone, but it is very useful for completing the hardening of

such organs as the liver, kidney, intestine, etc., when these have been previously partially hardened by the agents just mentioned.

279. **Chromic Acid and Alcohol.**—A solution of chromic acid in dilute alcohol is prepared thus :—

Chromic acid, 1 gramme.

Water, 20CC.

Dissolve, and slowly add rectified spirit, 180CC.

This fluid, devised by my former assistant Dr. Pritchard, is excellent for hardening the retina, membranes of the cochlea, skin, and possibly some other organs. (pp. 4, 5, and 6).

280. Sections of organs hardened in the above substances generally require to be rendered transparent. Glycerine, or its diluted form—Farrants' solution, clove oil, or turpentine, are commonly employed, and the tissues are preserved in glycerine, Farrants' solution, or dammar.

281. **Osmic Acid** (OsO_4) is a very valuable hardening agent introduced by Max Schultze. It occurs in commerce in small vitreous-like masses kept in sealed tubes. It is most conveniently kept as a 1 per cent solution in distilled water, preserved in well-stoppered bottles protected from the light. It is excessively poisonous, and its disagreeable, heavy, pungent vapour is dangerously irritating to the conjunctiva and nostrils. Employed in solutions of $\frac{1}{10}$ to 1 per cent it is of great service for hardening embryonic tissues, nerve fibres, retina, connective tissue corpuscles, epithelium, and epidermis, testis, etc. It fixes the tissue elements without producing a granular precipitate or causing shrivelling. It blackens fat and the medullary matter of white nerve fibres, and it also darkens, though to a much less extent, albuminous tissues. From ten minutes to two days are required. The pieces of tissue should always be small. After hardening they are washed in distilled water, and are then placed in rectified spirit, or sections are made and mounted in a saturated solution of potassium acetate, or in Farrants' solution. The former is the best (Schultze), for glycerine renders them too transparent.

281A. **Silver Nitrate, Gold Chloride, Palladium Chloride,** are also used for hardening tissues, but they will

be referred to under the head of staining agents (§§ 327, 330, 331.)

282. **Freezing** is a method of very great value. With its aid, lung, kidney, spleen, lymphatic gland, vitreous humour, brain, etc., may all be removed from the living animal, frozen, and sliced, within fifteen minutes.

Freezing is suitable for hardening the brain and spinal cord, *only* when slices of the fresh tissue are desired for the isolation of nerve cells, etc. These organs are best hardened in chromic acid, potassium bichromate, etc., not because they can be more readily cut when hardened by these substances, but for the reason that, when the tissue has been previously hardened by these agents, the slices do not go to pieces when they are manipulated, as they are apt to do when the *unhardened* frozen tissue thaws after freezing. For the same reason, it is advantageous, in the case of the kidney, lung, liver, spleen, and such like, to have the process of hardening carried to some extent by Müller's fluid, potassium bichromate, chromic or picric acid, before freezing them for the purpose of section. The special cases where freezing is of service have been indicated in the preceding demonstrations.

Indeed, speaking generally, freezing is of most service —1. *In enabling us to quickly harden and cut fresh tissues when the permanent preservation of these is not a primary consideration.* 2. *In completing, with a view to section, the hardening of tissues already partially indurated by other means;* so that—*a.* The process of hardening is shortened; *b.* The tendency to shrivelling of the tissue by the hardening fluid is lessened, because of its shorter exposure to its influence; *c.* The sections, when they thaw, are still sufficiently indurated by their treatment previous to freezing, to permit of their successful manipulation and preservation. Freezing may be employed after any of the hardening agents described in the preceding paragraphs, but in the case of alcohol, it of course must be completely removed by soaking the tissue in water ere the freezing can be accomplished.

Freezing has hitherto been had but little recourse to,

owing to the clumsy and inconvenient method hitherto adopted. This has simply consisted in placing the tissue on tinfoil, or in a platinum capsule, and setting it in a freezing mixture. It is very difficult and tedious to freeze any large piece of tissue in this way, and when it is removed from the freezing mixture, for the purpose of section, it begins to thaw, and thereby gives rise to so much inconvenience, and to results so unsatisfactory, that the method of freezing has been almost neglected. The method is now, however, rendered simple and satisfactory with the aid of the freezing microtome (Fig. 58). It is convenient to study its mode of employment in § 302.

METHODS OF SOFTENING THE TISSUES.

283. **Removal of Calcareous Matter.**—*Chromic and nitric acid* both harden albuminous tissues, but soften those which owe their hardness to the presence of calcareous salts. Bone and tooth may be softened in the following fluid :—

Chromic acid, 1 gramme.
Water 200CC. Dissolve and add
Commercial nitric acid, 2CC.

This chromic and nitric fluid gives far better results than dilute nitric or hydrochloric acid used alone, for while the nitric acid more especially removes the calcareous matter, the chromic acid especially hardens the bone protoplasts. This fluid produces excellent results. For details, see § 15.

Small pieces of bone may also be softened in a $\frac{1}{2}$ per cent solution of *chromic acid*, or in a solution of *picric acid*, kept saturated by the presence of superabundant crystals. The latter is preferable. This method is useful for fœtal bone (§ 119).

284. **Softening of Connective Substances.**—*Dilute alcohol* (rectified spirit * 1 part, water 2 parts) is of much

* Ranvier recommends alcohol at 36° of Cartier; this is very nearly the strength of ordinary rectified spirit, the sp. gr. of which is 0·838.

service for softening white fibrous tissue (*Ranvier*), when, *e.g.*, it is desired to separate muscular fibres. It also softens the cementing matter between epithelial cells, and thus permits of their ready isolation.

285. *Boiling*—by converting white fibrous tissue into gelatine—is useful for permitting of the isolation of muscular fibre in the making of coarse dissections.

286. *Dilute sulphuric acid.*—White fibrous tissue is rapidly dissolved by *maceration*, at a temperature of 46°C., in dilute sulphuric acid (commercial acid 1 part, water 1000 parts). This method is very useful in making coarse dissections of muscle. The cement connecting the fibres of the lens may be dissolved by *boiling* for five or ten minutes in dilute sulphuric acid (1 per cent).

287. *Acetic acid and glycerine* (glycerine 1 ounce, glacial acetic acid 5 drops) is recommended by Beale for softened white fibrous tissue, in tracing the course of nerve fibres with high powers. The white fibrous tissue is rendered very transparent and gelatinous, while medullated nerve fibres are rendered *granular*. In this way the nerve elements are readily recognised. This mode of tracing fine nerves has now, however, been largely superseded by the method of staining them with chloride of gold.

288. *Iodised serum* (§ 267) is recommended by Schultze for the maceration of white nerve fibres. The cement between the fibrils is softened and dissolved.

289. *Chromic acid, potassium bichromate.*—Although these substances have already been described as hardening agents, they are also used for softening connective substances. $\frac{1}{4}$ per cent solution of chromic acid softens the material at the sides of the prisms of striped muscle, and so facilitates cleavage into fibrillæ (§ 141).

A very dilute solution of chromic acid (one or two parts to 10,000 of water) is also an excellent macerating agent for the separation of epithelial cells. So also is a very dilute solution of potassium bichromate (one or two parts to 1000 of water). These very dilute fluids should have a few drops of carbolic acid added to prevent putrefaction of the tissue.

289A. **Method of Digestion.**—Artificial digestion in a fluid containing pepsine and hydrochloric acid has been used for causing striped muscle to cleave into discs. It may also be employed for softening and clarifying white fibrous tissue. The manner in which it may be applied to the skin in studying the arrangements of its elastic fibres is detailed in § 223 *b*.

MECHANICAL DISSOCIATION OF TISSUE ELEMENTS.

290. Tissue elements may be separated by various mechanical methods.

a. By dissection with needles.—Those most suitable are thick sewing needles fixed in strong handles (Figs. 54 and 34).

FIG. 54.—Dissecting needle.

Delicate textures and organs should be dissected under fluid.

b. By shaking.—This is a most useful method of dissociation, *e.g.*, the lymph corpuscles may be readily freed from the adenoid tissue in a section of lymphatic gland in this way (§ 110). The shaking is performed in a test tube containing some desirable fluid.

c. By pressure under the cover-glass, one is often able to separate tissue elements so as to display them effectively. The mode of demonstrating nerve cells in the fresh brain, detailed in § 247, *c*, is a good example of this method, and there are many others with which every one who works with the microscope for some time, soon becomes familiar.

It will be readily understood that the various fluids for softening connective substances facilitate the *mechanical* dissociation of tissue elements.

METHODS OF MAKING SECTIONS.

291. **Saw.**—Sections of unsoftened bone are made with a fine saw. The scratches thereby produced, are got rid of, and the sections at the same time ground sufficiently thin upon a stone, after the manner already described (§ 118).

292. **Scissors** are in almost constant requisition for removing small portions of soft tissues for microscopical preparation. The most convenient form is that shown in Fig. 55.

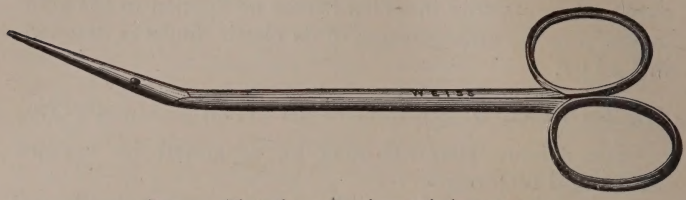

FIG. 55.—Fine scissors for microscopical purposes.

293. **Valentin's Knife** was formerly much employed for making thin slices of organs, such as the kidney, lung, stomach, etc., in the fresh condition. It consists (Fig. 56) of two parallel blades. In using the knife, these are first clamped in close apposition by the wedge *a*. They are then opened to any desirable distance by the screw *b*, and are, of course, fixed by the forces of the wedge and the screw acting in opposite directions. The thickness of the

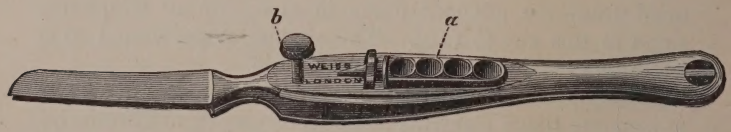

FIG. 56.—Valentin's knife.

slice depends on the distance between the blades. The sections obtained by this knife are far inferior to those made with a freezing microtome, but nevertheless it is useful when the microtome cannot be employed.

294. **Single-bladed knives.**—A *razor* and a *scalpel* are the knives most commonly used for cutting any tissue which by softening or hardening has been brought to a suitable consistence. The "*army* razors," made by Heifor of Sheffield, are the most convenient for all ordinary purposes. They are made of *good* steel—a most essential point—and their blades are thin and broad enough to give good sections. Similar razors, made for section-cutting by Weiss of London,

are still better. They are, however, only suitable for making sections of tissue held in the hand, their blades being too thin for use with a microtome. For ordinary purposes it is not necessary to have the razor ground flat on one side —(the right when the face is down). It does well enough to have the blade concave on both sides, provided it be broad enough to receive the whole section ere it come against the thick back of the blade. If one attempt, however, to make large sections without the aid of a machine, then a long broad-bladed knife, flat on both sides is necessary, but it is only a waste of time to attempt anything of the sort without a microtome for the purpose of keeping the tissue fixed and the blade gliding steadily and evenly during the process of section (see § 302).

Beer's knife is a useful instrument for puncturing the cornea when a drop of aqueous humour is wanted. An ordinary scalpel is not suitable for this purpose. Beer's knife has a thin triangular blade tapering to a fine point. It may also be used for making sections in place of a scalpel.

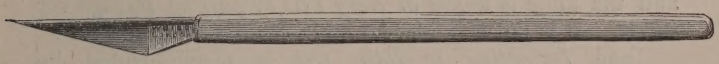

FIG. 57.—Beer's knife.

295. **Wetting the Knife.**—The knife *must always be wet, in order that the slice of tissue may float over its surface. a Water, b salt solution, c methylated or rectified spirit, d absolute alcohol*, are used for this purpose. Of these, *c* and *d* are the best, but they are only to be used in cases where spirit may be added to the tissue without altering it—*e.g.*, when the tissue has been hardened in spirit, Müller's fluid, or chromic acid.

296. **Hand-sections.**—If the tissue and the knife be both simply supported by the hands, the sections are, for convenience, often termed *hand-sections*. The tissue is held between the thumb and fore and middle fingers of the left hand, and the forefinger is used as a table for supporting the back of the knife. The knife should *never be held like a pen*, and it should *never be pushed* but *always drawn* from heel to point obliquely through the tissue. The sec-

tions should always be manipulated with camel-hair pencils. Much practice is required before dexterity is attained, but it is very important to thoroughly practise this mode of section; for, when only small slices are required, they can be made very perfectly in this simple way.

297. **Imbedding.**—The piece of tissue is often so small that it is impossible to hold it in the hand, and it is sometimes so brittle that it is apt to go to pieces when cut. To overcome these difficulties, it is necessary to imbed the tissue, so as to support and hold it firm during the section.

298. *Imbedding in Carrot.*—A carrot is an excellent imbedding agent. For holding in the hand a piece of softened bone or hardened spinal cord, etc., it is extremely convenient. A hole rather smaller than the organ should be scooped in the carrot, near its centre. An incision should then be carried from the hole to the periphery, so that the hole may be enlarged for the admission of the organ, round which the carrot closes like a hollow spring. The carrot does not cling either to the knife or to the texture; and the knife is *wetted at every slice by passing through the carrot.* It is not suitable, however, for delicate tissues.

299. *Imbedding in elder pith* is recommended by Ranvier. A hole somewhat larger than the tissue is made in the pith, and the piece of tissue—*e.g.*, a hardened nerve—is placed in it. The whole is then immersed in water, to cause the pith to swell up and clasp the tissue firmly.

300. *Imbedding in Paraffin.*—Paraffin is the imbedding agent most commonly employed. Ordinary solid paraffin is too hard. Five parts of it should therefore be mixed with one part of hog's lard with the aid of a gentle heat. The addition of a little clove oil renders the mixture less liable to adhere to the knife. A mixture of equal parts of bees'-wax and sweet oil is often recommended, and no doubt it answers very well for hand sections; but it is not so suitable as paraffin for imbedding in a microtome, because the wax and oil, having a higher melting point than the paraffin mixture, contracts so much when it cools, that it separates from the metal around the well of the microtome.

Melt the paraffin mixture in a water bath, at as low a temperature as possible, pour it into a paper cone or paper saucer, or into the well of a microtome. With forceps, dip the tissue into the paraffin, at once remove it, and, when the layer of paraffin has cooled, replace it finally in the paraffin. By this means the overheating of the tissue will be avoided. Tissues to be imbedded in paraffin should be as dry as possible, otherwise the supporting substance will not cling to the tissue. The water may be removed from the surface of the tissue by immersing it for a short time in spirit, and then allowing it to dry.

The great fault of paraffin is, that its slices get in the way, and often spoil the slice of the tissue while it is being made; moreover, it is difficult to get rid of. The sections require to be picked out from its débris, or this must be washed away from them. This implies considerable loss of time.

301. *Imbedding in Gum.—a.* This method, which we owe to Brücke, is a very valuable one. The solution of gum should be as thick as possible. It is made by pouring cold water on "picked" (*clean*) gum-arabic, and stirring it occasionally. It is extremely useful for imbedding the lung, and other tissues of irregular configuration, where an interstitial and not merely an external support is necessary. *Any alcohol in the tissue must always be carefully removed* by immersion in water for twenty-four hours or so previous to placing them in the gum.

b. For imbedding the lung, a small piece that has been previously hardened by chromic acid in the usual way, and from which all spirit has been removed, is placed for thirty-six or forty-eight hours in a thick solution of gum. It is then transferred to methylated spirit, in which it soon becomes sufficiently hard to permit of easy section.

c. For the retina, cochlea, small intestine, and other parts with delicate tissues needing careful support, the following method is used:—Place the tissue in a cone of bibulous paper containing thick solution of gum for six to twenty-four hours. Then set the cone with its contents in methylated spirit for forty-eight hours or so, to remove the

water and so stiffen the gum. Lastly, remove the paper, imbed in paraffin, and make sections.

d. The gum may be readily removed from the sections by immersion for some time in water.

302. **Microtome.**—In making hand-sections with such a knife as a razor, success depends on the steadiness of the operator. It is, however, always difficult to hold the tissue and the knife perfectly steady, and when a large section is wanted—say of kidney, spinal cord, or brain—a great many

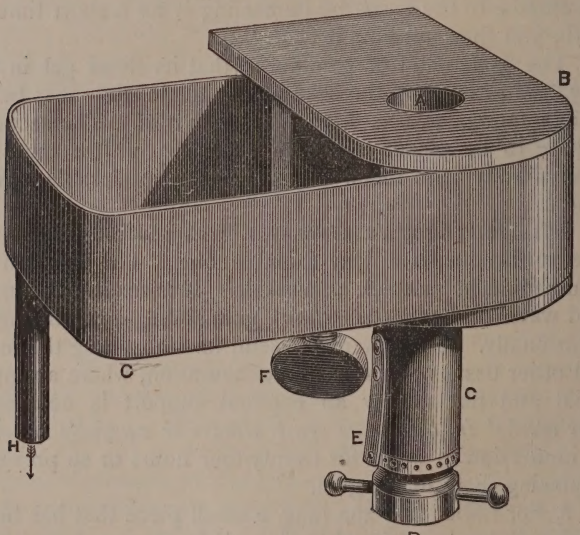

FIG. 58.—The freezing microtome.

sections have to be thrown away as imperfect. This difficulty is overcome by employing a microtome. Various microtomes have long been in use. These, however, it is unnecessary to describe, for the freezing microtome invented by the author presents advantages which were not possessed by any previous instrument.

The freezing microtome (Fig. 58) consists of a plate of gun-metal (B), with a circular opening in its centre. The opening leads into a well (A), closed inferiorly by a brass

plug (K, Fig. 59), capable of being moved up or down by means of a screw (D). The tissue to be frozen is placed in the well, and sections are made by gliding a knife through the tissue that projects above the level of the brass table.

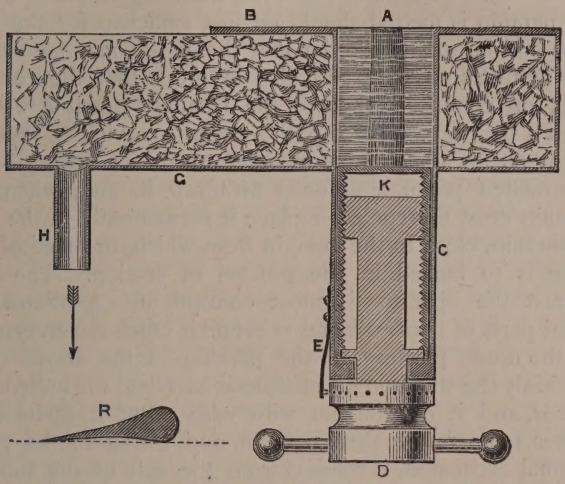

FIG. 59.—Vertical section of freezing microtome. R, Transverse section of the knife employed.

The thickness of the sections is regulated by an indicator (E). A freezing mixture is placed in the box (C), the water from which flows away by the tube (H). The microtome is clamped to a table by the screw (F). The tissue is seen in A, Fig. 59, and the freezing mixture in G of the same figure. In the instruments now made, the brass box is covered with gutta-percha.

The freezing microtome is essentially the well-known microtome devised by Mr. Stirling, Sub-Curator of the Anatomical Museum of the University of Edinburgh, modified for freezing. It serves a double purpose. 1. It serves the same purpose as the microtome of which it is a modification ; that is to say, it may be used for *cutting unfrozen tissues imbedded in paraffin in the usual way.* 2. For cutting tissues *hardened by freezing.* The second method of using

the machine will be more readily comprehended after a description of the first.

303. *When the microtome is used without freezing*, the tissue may be imbedded in paraffin or in carrot.

a. When paraffin is employed the temperature at which the paraffin is melted should be just sufficient for the purpose and no more, in order that the tissue may be heated as slightly as possible, and also in order that the paraffin may, on cooling, shrink to the smallest possible extent. With a pair of forceps dip the tissue—previously exposed to the air for a short time in order to dry its surface—into the melted paraffin, at once withdraw it, and, when the paraffin crust has cooled, replace it permanently in the well of paraffin, close to the margin from which the face of the knife is to moved in the process of section. The side nearest the operator is most convenient. A mixture of equal parts of bees'-wax and sweet oil is much recommended for the same purpose as the paraffin in the above case. Certainly the wax and oil mixture is excellent for imbedding tissues, and it can be cut with ease. But it melts at a higher temperature than paraffin, and, owing to the great thermal expansion, it retracts from the side of the tube of the machine, and so the wax cylinder becomes loose. The only way in which this can be prevented is by heating the machine to a like temperature before introducing the wax. This is tedious; and, inasmuch as it is unnecessary in the case of paraffin, this is to be preferred. Even with this, however, the paraffin cylinder is apt to become a little loose, and to turn round in the machine: hence it is important that there be an *eccentric* hole in the brass plug (K, Fig. 59) with a projecting wooden pin fixed in it. The rotation of the paraffin cylinder is thus prevented.

b. When carrot is used a cylinder of it to fit the well of the microtome must cut with a cork-cutter, and the tissue imbedded as already described in § 298.

304. The knife must have a back with straight edges, so that it may not tilt in the process of section. The surface that rests on the plate should always be hollow, and it is convenient to have the upper surface the same (R, Fig. 59),

unless the sections be very large, when it should be flat or slightly convex. The concavity is convenient for carrying a pool of spirit, over which the section *floats ; the floating is essential.* It is convenient to keep the back of the knife towards the operator, and either to *draw* it steadily through the tissue from heel to point, or to *push* it in the opposite direction. The blade of the knife should not be too thin, otherwise it tends to bend into the well. A considerable amount of dexterity is required, but this is soon gained by practice.

305. *When the microtome is used for freezing*, the freezing box requires to be covered with a non-conductor, such as a thick jacket of felt, flannel, or gutta-percha. The latter is much to be preferred, and an instrument having this covering should be obtained. The plug (K, Fig. 59) is unscrewed and oiled to prevent its becoming fixed during the freezing. The tissue and an imbedding fluid are placed in the well. If a watery fluid be employed, it becomes crystalline when frozen, and is therefore badly adapted to serve as the imbedding agent.

306. The difficulty is, however, entirely overcome by the method of imbedding in gum (§ 301), as suggested to me by my late assistant, Dr. Pritchard. A *thick* solution of gum when frozen does not become crystalline, and can be *cut like cheese.* If there be any alcohol in the tissue it must be thoroughly removed by immersion in water for six to twenty-four hours, according to the size of the tissue, before it is placed in the gum. *It is always advantageous to allow the tissue to soak in the gum for twelve to twenty-four hours before freezing, in order that the gum may permeate every part of the tissue and prevent the formation of a crystalline condition within the frozen tissue.* Thick gum solution is poured into the well, and, when a film of ice has formed at the periphery, the tissue should be introduced and held against the advancing ice until it is fixed. In this way the tissue may be secured in any position for the process of section. A plate of cork with a weight on it is placed on the microtome plate to prevent the entrance of heat, and also of salt from the freezing mixture.

307. Small quantities of *finely* powdered ice and salt are placed alternately with the aid of a small spoon in the freezing box, and are stirred round the well, the tube H being carefully kept open to allow the water to escape from the melting ice. When snow can be obtained, it is, of course, used instead of ice. The powdering of the ice is rather tedious, but it may be readily done in that form of sausage machine where two cylinders with spiral grooves are rolled against one another. Before putting the ice into this apparatus it should be wrapped in a thick cloth and broken with a wooden mallet. The freezing process is really very simple, and can be fully carried out in from ten to twenty minutes. Of course the period is shorter the colder the surrounding air.

308. It is not necessary to wet the knife, for this is thoroughly done by the thawing gum. The sections often contain a number of air bubbles; these, however, together with the gum, are soon removed by placing them in dilute alcohol (rectified or methylated spirit 1, water 2 parts). If the sections are to be preserved for a time until they are examined or mounted, they are transferred from the dilute alcohol to rectified spirit. When sections are apt to be spoilt by manipulation, *they should be placed on the slide before they thaw.* They can then be readily washed by making a pool of dilute alcohol around them, which may be changed two or three times.

309. The more one employs the freezing microtome, the more clearly does one perceive the great assistance which it offers to the histologist. A number of tissues may be frozen and cut at the same time. Delicate organs, such as the retina, the embryo, villi of the intestine, lung, trachea with its ciliated epithelium, may all be readily cut without fear of their being destroyed by the imbedding agent, as is apt to be the case when paraffin, pith, and carrot are employed. The cases to which freezing is applicable have already been detailed in § 282. It must be carefully borne in mind, however, that in the case of a tissue saturated with *water* spicules of ice are formed, and after section various crevices may sometimes be seen which have resulted from

these spicules, or from the laceration of the tissue due to the knife having come in contact with them. This difficulty is *overcome by a thorough saturation of the tissue with gum* previous to freezing, as described in § 306.

310. *Alterations of the freezing microtome.*—The freezing microtome was invented and first described by me in the *Journal of Anatomy and Physiology*, May 1871. The instrument there figured was simply a Stirling's section-cutter, to which I adapted a freezing box. I soon found, however, that the box was too small, the well too large, and the screw imperfect. I therefore altered the apparatus, and described the new form (Fig. 58) in the *Lancet* (July 1873). The only further alteration that I have made is to have the freezing box entirely covered with gutta-percha, the escape tube (H, Fig. 58) made larger, and the cutting-table made longer between the well and the end nearest the operator. These are real improvements, which make the instrument work in a most satisfactory manner.* At the same time, it may be stated that the identical instrument described in the *Lancet* has been in daily use in my laboratory for three years. We use no other, because it really has always answered the purpose remarkably well. It has often been suggested that the freezing box should project at the right as well as at the left side of the machine. This arrangement I have carefully avoided from the first, because it interferes with the hand in using the knife, and certainly it is not necessary.

METHODS OF INCREASING THE TRANSPARENCY OF THE TISSUES.

311. The transparency of the tissues may be increased —1. By impregnating them with fluids which strongly refract light, *e.g. glycerine*, *turpentine*, *clove oil*, *Canada bal-*

* The instrument thus modified is made by Mr. Gardner, instrument maker, South Bridge, Edinburgh, and I can no longer recommend the instruments of other makers, for they have shown themselves much more disposed to supply the instruments for which they happen to have had "castings" made than to adopt my suggestions.

sam, *dammar*. 2. By partially or completely dissolving certain elements of the tissues, so as to permit of others being seen. *Acetic acid*, *caustic potash or soda*, are used for this purpose. These partially or completely dissolve the soft albuminous parts of most tissues.

312. *Glycerine and acetic acid* may be used together as recommended by Beale. (Glycerine 1 oz., glacial acetic acid 5 drops.)

313. *Acetic acid.*—The acid most commonly employed is the ordinary pyroligneous acid. It contains 28 per cent of anhydrous acid (or 33·3 per cent of glacial acid). It is important for ordinary purposes not to employ the acid in too concentrated a form. Pyroligneous acid 1 part, water 2 parts, is the dilution most useful for ordinary purposes, but so great a dilution as 4 or 5 drops to an ounce of water is sometimes very serviceable, *e.g.* for showing the nerve terminations in striped muscle (§ 160, *Kölliker*) and for slowly softening white fibrous tissue.

314. *Caustic potash* is sometimes used for rendering albuminous tissues transparent, and for softening connecting substances. A 30 per cent solution of the caustic alkali in distilled water is the most generally useful solution (*Moleschott*). Ewart and Thin (*Journ. of Anatomy and Physiology*, vol. x. p. 223) have recently recommended a solution of caustic potash in an equal weight of distilled water as a means of preparing the fibres of the crystalline lens.

315. *Glycerine*, *acetic acid*, and *potash*, being all miscible with water, may be employed to clarify tissues from which water has not been removed. *Oil of cloves*, or other essential oil, *turpentine*, *creosote*, *carbolic acid*, render most tissues transparent. As these do not mix with water, it must be previously removed by drying the tissue, or by immersion in alcohol, and then allowing the alcohol to escape.

316. Tissues hardened in chromic or picric acid, and in potass. bichrom., are rendered somewhat opaque, and must in general be clarified. This is usually done by adding *a*, *clove oil*; *b*, *turpentine*; *c*, *glycerine*. These fluids have different powers—*a* is stronger than *b*, and *b* is stronger than *c*. Glycerine is employed for tissues that

must not be made too transparent, *e.g.* unstained spleen, alimentary canal, lung, liver. Generally speaking, clove oil and turpentine are too powerful as clarifying agents for tissues not stained with such dyes as carmine and logwood. When glycerine is used, the tissue is preserved in it. When clove oil or turpentine is employed, the tissue is preserved in dammar or Canada balsam.

METHODS OF STAINING THE TISSUES.

317. In staining the tissues, there are two points to be especially borne in mind. 1. The staining fluid may affect some parts of the tissue more than others, or it may stain some parts, and others not at all; *e.g.* the nucleus is more deeply tinged with carmine or magenta than the surrounding protoplasm, epithelial cement is deeply stained by silver nitrate, nerve fibrils by gold chloride. 2. The staining fluid may affect the tissue with tolerable uniformity, and prove serviceable *by merely rendering very transparent colourless parts more evident.*

318. **Carmine.**—The use of this valuable dye for histological purposes was first recommended by Gerlach. It tinges protoplasts, the axial cylinders of white nerve fibres, but not the *modified* protoplasm of which cilia and muscular fibre consist. It is useful to have two fluids.

a. Weak carmine fluid, an exceedingly good carmine fluid for general purposes, is a modification of that proposed by Beale. Beale's fluid is thus prepared :—

Carmine . . .	10 grains.
Strong ammonia . .	30 minims.
Glycerine . . .	2 ounces.
Rectified spirit . .	½ ounce.

Place the carmine in a test tube, add the ammonia, boil for a few seconds, let the solution stand for an hour with the test tube open, add the water, filter, add the spirit and the glycerine, and allow the mixture to stand exposed to the air until the odour of ammonia is scarcely perceptible, then keep it in a stoppered bottle. This fluid is, owing to its density, slow in its action, and, although it

may yield excellent results, it is very uncertain in its operation. Tardiness and uncertainty of action may be avoided *by retaining all the ammonia in the fluid*, and by diluting the fluid when it is to be used. Therefore, prepare the fluid thus :—Place the carmine in a test tube, add the ammonia, bring to the boiling point, add the water and filter, then add the spirit and glycerine, and preserve in a stoppered bottle. When about to be used it should be diluted from *two* to *seven* times with water. The last is the most generally useful dilution. The fluid should always be filtered after dilution, and the tissues placed in a large quantity of it from 2 to 24 hours. This fluid is therefore not at all suitable for rapid staining, but carmine staining is generally most beautiful when it takes place slowly.

b. Strong carmine fluid.—It is useful to have a strong carmine fluid for rapidly staining nerve tissues. It is thus prepared :—

Carmine . . .	1 gramme.
Strong ammonia . .	1CC.

Dissolve in a test tube, with the aid of a gentle heat, and add

Distilled water . .	23CC.

Filter and preserve in a stoppered bottle. For ordinary purposes it tinges too rapidly, and for tissues unhardened in chromic acid or Müller's fluid it contains too much ammonia. Therefore, should this fluid be used for staining in the ordinary way, expose it in a beaker to the air for two days, to let the excess of ammonia escape, then dilute it with five times its bulk of distilled water, filter, and preserve in a stoppered bottle.

319. *Carmine staining with palladium.*—When tissues, and especially nerve tissue, have been overhardened in chromic acid, carmine may entirely fail to stain them. In such a case the following method (*Merkel*) is of much service. Place a large drop of $\frac{1}{4}$ per cent watery solution of palladium chloride on a slide, and on another slide a large drop of a strong ammoniacal solution of carmine (§ 318, *b*). Allow the section to remain in the palladium for about two minutes. Wash it in water and place it in the carmine fluid for about three minutes. Then wash in water.

320. Carmine is, however, a somewhat uncertain dye, and it is often difficult to get good results, especially with tissues hardened in chromic acid. This substance, together with picric acid, potassium bichromate, and Müller's fluid, must always be removed by washing in water previous to staining.

321. After staining, the superfluous pigment is removed by washing in water acidulated with 1 per cent hydrochloric or glacial acetic acid, or in rectified spirit 60 parts, water 39 parts, hydrochloric acid 1 part (*Pritchard*). The acid brightens the colour.

Tissues stained with carmine may be mounted in Farrants' solution, glycerine, or dammar.

322. **Picro-carmine.** — The picro-carminate of ammonia introduced by Schwarz and Ranvier, is an excellent staining agent, and is prepared thus :—Take 100CC of a saturated solution of picric acid. Prepare an ammoniacal solution of carmine by dissolving 1 gramme in a few CC water, with the aid of an excess of ammonia and heat. Boil the picric acid solution on a sand-bath, and when boiling add the carmine solution. Evaporate the mixture to dryness. Dissolve the residue in 100CC water, and filter. A clear solution ought to be obtained ; if not, add some more ammonia, evaporate, and then dissolve as before.

A double staining is produced. Nuclei are stained by the carmine, while the picric acid gives a yellowish tinge to muscle, epithelium, and epidermis, etc. Sections of skin may be beautifully stained by this fluid : the epidermis, hair, and muscles of the hair follicles are rendered yellow, while the cutis vera is reddened. If carmine staining alone be desired, the stained tissues are placed in distilled water, by which the yellow colour from the picric acid is speedily removed.

323. **Logwood** is, like carmine, suitable for staining tissues for permanent preparations. Generally speaking, it stains the same tissue elements as carmine. The tint is violet, and on this account it is not nearly so fatiguing to the eye as a red dye. The tissues may either be fresh, or they may have been hardened in chromic acid or alcohol. It

stains the nuclei of epithelium, and the protoplasts of connective tissue remarkably well; nerve cells and axial cylinders, the lung, skin, cornea, tongue, etc., can be beautifully tinged by this agent. It is, however, a somewhat perplexing substance, on account of the difficulty experienced in getting a staining fluid capable of giving a pure violet tinge.* It was introduced by Boehmer. Kleinenberg's receipt for the solution is the best (Foster and Balfour's *Elements of Embryology*, p. 248).

A. Make a saturated solution of crystallised calcium chloride in alcohol (70 per cent), and add alum to saturation.

B. Make a saturated solution of alum in alcohol (70 per cent).

C. Add A to B in the proportion of 1:8.

D. Make a barely alkaline saturated solution of hæmatoxylin in water.

For staining place eight or ten drops of D in a watch-glass half filled with C; allow the tissue to remain in it until stained of sufficient depth; then wash in water. Diffuse staining may be removed by immersion in rectified or in methylated spirit, or in a $\frac{1}{2}$ per cent solution of alum.

Tissues stained with logwood are mounted in Farrants' solution, glycerine, or in dammar.

324. **Magenta** (rosaniline nitrate) is an extremely useful dye, because of its rapidity of action and the sharp definition which it gives to objects. Like carmine, it stains protoplasts, the axial cylinders of white nerve fibres, etc. Unlike carmine, it fades, and is therefore not suitable for permanent preparations. Nevertheless, the colour can be retained for a long time by mounting the stained tissue in one-third per cent watery solution of corrosive sublimate. Two fluids are necessary.

a. Ordinary magenta fluid prepared thus—

Magenta crystals . .	1 Decigramme.
Rectified spirit . .	9CC.
Distilled water . .	213CC.

* A good logwood staining fluid may be obtained from Martindale, chemist, New Cavendish Street, London, W.

Dissolve the magenta in the water, and then add the spirit. Preserve in a stoppered or well-corked bottle. This fluid is used for the tissues generally.

b. Strong magenta fluid prepared thus—

Magenta crystals . .	1 Decigramme.	
Distilled water . .	15CC.	Dissolve and add
Rectified spirit . .	5CC.	
Glycerine . . .	20CC.	

This fluid, devised by my former assistant, Dr. Ferrier, is used for staining blood corpuscles. Being of a specific gravity, similar to that of the liquor sanguinis, the coloured corpuscles of non-mammalian vertebrates alter but little in shape while they become stained.

325. **Aniline Blue-Black** * has been recently recommended by Mr. Sankey (*Quarterly Microscop. Journ.*, vol. xvi. p. 95) for staining the nerve cells of the brain and spinal cord. The tint which it gives to them is a blue-gray. He gives the following formula :—Dissolve 5 centigrammes of the dye in 2CC water, pour it into 99CC methylated spirit, and filter. The sections of brain stain in a few minutes. The sections are clarified with clove oil and mounted in dammar in the usual way. Mr. Lewis (*Quarterly Microscop. Journ.*, p. 73) recommends deep staining with an *aqueous* solution of the dye ($\frac{1}{4}$ to 1 per cent), and the subsequent removal of superfluous pigment, by immersion for twenty minutes or so in an aqueous solution of chloral hydrate (1 to 10 per cent). The chloral is then removed by washing in water, and the sections mounted in dammar (see § 247, *c*).

326. **Madder.**—Under the title "purpurine" the following fluid is recommended by Ranvier for staining cartilage cells :—Add 1 gramme of alum to 200CC distilled water ; bring them to the boiling point in a porcelain basin, and then add "some" extract of madder previously powdered, and rubbed up with a little water. The ebullition is continued, and a portion is dissolved. It is filtered while

* This is a cheap substance, and may be obtained from Messrs. Read, Holliday, and Sons, Huddersfield, or at 15 Fenchurch Street, London.

hot, and the filtrate is allowed to trickle into 60CC rectified spirit. Slices of cartilage are allowed to remain in the fluid from 24 to 48 hours. They are then washed in water, and mounted in glycerine. The nuclei and nucleoli of the cells are brightly coloured, the cartilage matrix faintly so. He also recommends its use in studying intra-cartilaginous ossification. It stains tissues hardened in chromic acid.

327. **Silver Nitrate.** — His, but more particularly Recklinghausen, introduced this substance into histology. It is of great value for staining the epithelium of serous membranes, lymphatics, and blood-vessels. It also stains the matrix of connective tissue and of cartilage, and often the nuclei of cells. In addition to staining, it fixes albuminous matter, and is thus valuable by facilitating the preservation of delicate tissues, *e.g.*, adenoid tissue of lymphatic gland, epithelium of intestine. A solution of the salt is commonly used, but the solid form is also sometimes employed for the cornea.

328. *The ordinary silver process.*—A $\frac{1}{2}$ per cent solution in distilled water is usually employed, but somewhat weaker solutions may also be used. In silvering a tissue—*e.g.*, the mesentery of the frog, or omentum of guinea-pig or cat—dip it two or three times into distilled water to remove sodium chloride. Place it in the silver solution from one to three minutes. Remove the superfluous silver by washing in distilled water. Place the washed tissue in glycerine or in distilled water, but glycerine is better, and expose it to *diffuse* daylight until it becomes slightly brown. Mount in glycerine, or in glycerine jelly.

By exposure to light the silver salt is reduced, and the tissues are thereby blackened. If the immersion of the tissue in the silver solution be of very brief duration, only the outlines of the epithelial cells are blackened. The cells appear to be united by a colourless "cement," which readily reduces the silver salt when acted on by light. If the silver act for some time longer, the nucleus and general substance of the epithelial cells is blackened, also the nuclei of fat cells, connective tissue corpuscles, etc.

Tissues that have been slightly silvered may be stained with logwood or carmine; the ammonia of the latter, however should be neutralised with acetic acid, and the tissue washed in acidulated water (§ 321).

329. *Silver process for blood-vessels.* If a frog be taken, kill it by stunning it on the head: expose the heart, snip off its apex, and allow it to bleed thoroughly. Push a glass or brass cannula from the ventricle into one of the aortæ, and inject a stream of distilled water to wash out the blood with its chlorides: inject $\frac{1}{4}$ per cent silver nitrate solution, allow it to remain for 8 or 10 minutes, and then wash it out with distilled water. The most convenient parts to take are the mesentery and bladder. These are therefore removed and exposed to the light, as stated in § 328. If, however, it is desirable to silver also the epithelium covering the mesentery, this is done after the injection, as described in § 328. In the case of a warm-blooded animal, such as a guinea-pig, the water and the silver solution are both heated to 39° C. The fluids are injected into the aorta.

A solution of silver nitrate may also be injected into lymphatic gland, areolar tissue, testis, lung, etc., for the fixing and staining of tissue elements. The necessary apparatus for injection is described in § 344.

330. **Chloride of Gold** (auric chloride) is of much service for staining nerve fibrils, connective tissue corpuscles, cartilage cells, etc. It is not merely a staining, but, like silver nitrate, is also a hardening agent. Unfortunately its action as a dye is somewhat uncertain.

a. Ordinary Method.—Place the tissue, not later than fifteen minutes after its removal from the body, in $\frac{1}{2}$ per cent solution of auric chloride in distilled water, until it becomes of a pale yellow colour. From fifteen minutes to two hours will be required, according to the thickness of the tissue. Transfer to very dilute acetic acid (1 or 2 per cent) for five or ten minutes. Lastly, place the tissue in distilled water, and expose to diffuse daylight, until a steel-gray or violet tint appear. This results from the reduction of the gold salt. The reduction is hastened by the acetic acid. The tissue may also be exposed to the light in dilute

acetic acid or in glycerine. The time of exposure varies with the intensity of the light; from one to two days usually suffice. Mount the stained tissue in glycerine, or in glycerine jelly. The preparations deteriorate however.

b. Method for the Cornea.—The gold method was introduced by Cohnheim for staining the nerves of the cornea. The following method, devised by Klein (*Monthly Microscopical Journ.*, vol. vii. p. 157), gives better results than Cohnheim's in this case. Remove the cornea from a rabbit or guinea-pig within fifteen minutes after death, and place it in $\frac{1}{2}$ per cent gold solution, from one and a half to two hours in the case of the rabbit, and rather more than an hour in the guinea-pig. Wash in distilled water, and expose to the light in the water for twenty-four or thirty-six hours, the water being changed twice or thrice. Then transfer the cornea to dilute glycerine (pure glycerine 1 part, distilled water 2 parts) for two or three days. Lastly, place the cornea in water, and gently brush away any precipitate that may have formed; make sections obliquely horizontal or vertical, and mount in glycerine. A cornea so treated is ash-gray or slightly violet.

331. **Chloride of Palladium** was introduced by F. F. Schultze for staining non-striped muscle. It renders the fibres brownish yellow. An aqueous solution of 1 per 1000 is employed, to every 20CC of which a drop of hydrochloric acid is added, in order to preserve it. For staining, three or four days are required. Schultze recommends this as a hardening agent for the lens and the retina. Its use as an adjuvant in the carmine staining of nerve tissue hardened in chromic acid has been alluded to in § 319.

332. **Osmic Acid.**—The value of this substance for blackening, more especially medullary substance of nerve and all fatty matter, has been already alluded to in § 281, where it has been described under the head of hardening agents, because of its especial value as such.

METHODS OF INJECTION.

I.—INJECTION MASSES FLUID AT THE ORDINARY TEMPERATURE.

333. **Aqueous Solution of soluble Prussian Blue.**—Soluble Prussian blue can now be procured in the market. The following, however, is the method in which it may be prepared (Brücke).

A. Dissolve 217 grammes potassium ferrocyanide in 1 litre distilled water.

B. Dissolve 100 grammes ferric chloride in 1 litre distilled water.

C. Make a cold saturated solution of sodium sulphate.

Mix 1 volume of A with two volumes of C, and mix 1 volume of B with 2 volumes of C. Pour B C very gradually into A C, stirring all the while, and let stand for a day. Pour off supernatant fluid, and filter off the remaining fluid from the precipitate. Wash the precipitate upon the filter, with repeated small quantities of distilled water, until it flows through distinctly blue. Press and dry the deposit.

For the purpose of injection, a 2 per cent solution of the blue in distilled water is employed. The fluid may be used cold, or it may be heated to the temperature of the body. After injection, prevent the blue from diffusing through the capillary walls by immersion in spirit (90 per cent). If the colour fade, immersion in turpentine, especially in that which has been exposed to the air for some time, or in acid, restores it. Mount in dammar; glycerine must be avoided.

334. **Beale's Prussian Blue injection.**

A. Mix 1 oz. glycerine with 4 oz. water.

B. Add 60 minims tincture of ferric chloride (British pharmacopœia) to 1 oz. of solution A.

C. Dissolve 12 grains potassium ferrocyanide in 1 oz. of solution A.

Pour B into C very gradually, with constant stirring and

shaking of the mixture, then add the remainder of solution A, and also 1 oz. rectified spirit. The injected tissues are preserved in acid glycerine (glycerine 1 oz., hydrochloric acid two drops).

Prussian blue injections must not come in contact with alkali. The above injection masses are excellent when it is of no moment to have the vessels fully distended, but merely to have the indication of pigment within them, to aid in their recognition amidst other tissue elements.

334A. **Asphalt and Chloroform.**—Ludwig has recently employed a mass, consisting of asphalt dissolved in chloroform and filtered, for the injection of the bile ducts. The merit of this fluid is, that chloroform being an extremely mobile fluid, flows readily along the vessels, and that it rapidly evaporates and leaves them filled by a solid black mass.

335. **Alcannin and Turpentine.**—A solution of alcannin in turpentine or in chloroform is used by Ludwig for injecting lymphatics. The solution is of a bright red colour. Both the turpentine and the chloroform solutions flow readily. When the latter is employed, the chloroform evaporates and leaves the alcannin in the vessels.

336. **Solution of Silver Nitrate.**—The employment of this has already been referred to in § 329.

2.—INJECTION MASSES SOLID AT THE ORDINARY TEMPERATURE.

337. **Carter's Carmine and Gelatine Mass.**—This —the best of all the carmine masses—is prepared thus :—.

Carmine, 60 grains.
Strong ammonia, 120 minims.
Glacial acetic acid, 86 minims.
Solution of gelatine (gelatine 1, water 6 parts), 2 oz.
Water, 1½ oz.

Dissolve the carmine in the ammonia and water with the aid of gentle heat, and filter; add to this 1½ oz. of hot gelatine solution, and mix thoroughly. Add the acid to

the remaining ½ oz. of gelatine solution, and drop this into the heated carmine mixture with constant stirring. The acid is added in order to precipitate the carmine and prevent its diffusion through the walls of the capillaries. The presence of the gelatine prevents the precipitated particles from assuming a coarse granular form. For section, the tissues are hardened—first, in equal parts of rectified spirit and water, with the addition of 1 per cent hydrochloric acid; then in rectified spirit; and, lastly, in absolute alcohol. Sections are then made, and mounted in dammar.

338. **Prussian Blue and Gelatine Mass.**—

A { Gelatine, 33 grammes. Distilled water, 200CC. Dissolve with the aid of a gentle heat (using a water-bath).

B { Soluble Prussian blue, 4 grammes. Distilled water, 300CC. Dissolve.

Add B to A gradually. The tissues are hardened for section and preserved, as stated in § 337.

339. **Injection of Blood-vessels.**—*Syringe.*—A brass syringe S (Fig. 60), capable of holding from four to six

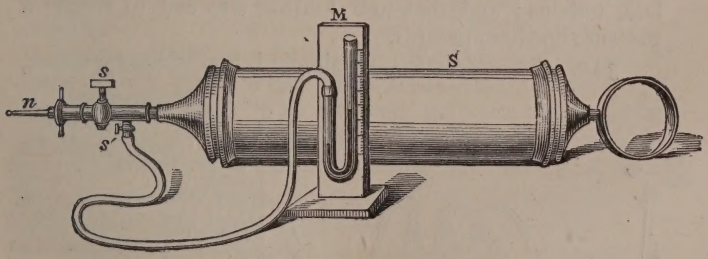

FIG. 60.—Injection syringe (S) with mercurial manometer (M).

ounces of fluid, is very often used for injection. It is usually provided with brass nozzles (*n*) of various sizes, for vessels of different calibre, and a stopcock (*s*) which may be separated from the nozzle and from the syringe. The nozzle is tied in an artery; the stopcock-piece is pushed into it; both are filled with injection-mass by means of a pipette; and the stopcock turned. The syringe is then filled—care being taken to expel all air by holding the

nozzle uppermost while a drop or two of injection is forced out. A few drops are allowed to fall into the stopcock-piece to completely fill it. The two are united, the stop-cock turned, and the injection slowly performed. The piston of the syringe must *never be pushed*, but always *screwed* down, to ensure steadiness of pressure. This apparently simple process is very apt to fail because of the difficulty of judging of the amount of pressure applied. If it be too great, capillaries are over-distended or ruptured, and the injection spoilt. This is easily avoided, however, by attaching—as is sometimes done—a pressure-gauge to the syringe. This is an ordinary mercurial manometer (M) connected to the stopcock-piece of the syringe by an elastic tube containing air, and a stopcock *s'*. When both stop-cocks are open, and the fluid expelled from the syringe, a part of it passes into the elastic tube, compresses the air, and elevates the mercury in the one limb of the manometer above that in the other, and thus indicates the pressure under which the fluid is being driven into the vessels. The syringe, though convenient for rapid injection, is, nevertheless, tedious; and therefore Ludwig was led to devise a pressure-bottle apparatus.

340. *Pressure-bottles.*—A simple but efficient apparatus,

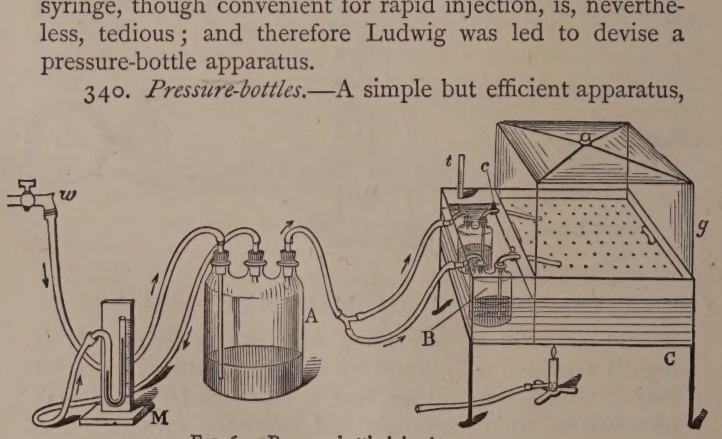

FIG. 61.—Pressure-bottle injection apparatus.

essentially similar to that used by Ludwig for prolonged injections, is shown in Fig. 61. *c* represents a zinc or copper water-bath with a perforated tray, on which the

animal or organ is laid, and covered with glass (*g*). The water is warmed by an ordinary Bunsen's burner, and its temperature is indicated by a thermometer (*t*). Two Wolff's bottles (B)—one containing, say a carmine mass for injecting arteries, and the other a Prussian blue mass for veins—stand in the water, so that when a hot injection has to be made, the mass may be readily warmed. The masses are expelled from them by air driven from a large Wolff's bottle A, by water flowing into it from a tap (*w*). The pressure exerted upon the air in A is indicated by a mercurial manometer M, and its amount, of course, depends upon the rapidity with which the water is allowed to flow. Glass nozzles, each with a small shoulder, as in *n*, Fig. 60, for securing them in the vessels, are tied in the indiarubber tubes from the injection-bottles (B); and when one or other injection is not required, it is cut off by the stopcock of its exit tube (*c*). The corks of the bottles are of vulcanised indiarubber. The lengths of the glass tubes penetrating them are indicated in the Fig. The elastic tubing requires to be very carefully tied over the glass to prevent leakage. Such an apparatus is of great service, for its action is automatic, and the pressure can be very finely graduated.

341. **Injection.**— The blood-vessels should be injected immediately after death from chloroform. When the breathing is just about to cease the right auricle should be opened to allow the vessels to empty. Previous to injection the vessels may be washed out with a stream of $\frac{3}{4}$ per cent salt solution, heated to 40° C. if the animal be warm-blooded. The use of the salt solution, however, is not essential.

If a gelatine injection be used, it must of necessity be heated to about 40° C., and the animal must also be kept warm; but even when an injection that is fluid at ordinary temperatures is employed, it should be heated 40° C. or so, because of the vascular spasm which a cold fluid is apt to occasion.

It is very important always to begin with a low pressure, say $\frac{1}{2}$ an inch of mercury, and to increase it very gradually to 3 or even to 4 inches. When the injection is complete the artery and vein are ligatured, and the organ placed

in cold methylated spirit, to cause the gelatine to consolidate rapidly, and so prevent its escape. After several hours, or a day, the preparation is removed from the alcohol, and treated as may be desirable.

342. For the injection of the *kidney* the organ should not be removed from the animal, because of the anastomoses between the renal and lumbar vessels. The nozzle should be placed in the renal artery close to the aorta.

343. For the injection of the *liver* from the portal vein, and the *lung* from the pulmonary artery, salt solution should first be injected, the right and left auricles of the heart being respectively opened to permit the blood to escape.

344. **Injection of Lymphatics.**—*The puncture method* for the injection of lymphatics was introduced by Ludwig, and simply consists in thrusting the sharp needle-like nozzle of a Wood's syringe (the syringe of Pravaz of continental writers) into various textures, and driving the fluid wherever it will go. *e.g.* In the case of the *testis*, if the nozzle be thrust through the tunica albuginea after death, and the injection driven into the gland substance, it very readily finds its way into the lymphatics, and runs along them even as far as the thoracic duct, which may be thus injected. The chain of lymphatics in the leg of the dog can be easily demonstrated by simply thrusting the nozzle of the syringe into the pad of the foot after death, and pushing the fluid onwards by compressing the limb from below upwards. The lymphatics of *mucous membranes* and aponeuroses may be injected in the same manner. A watery solution of Brücke's blue (§ 333), or the turpentine or chloroform solution of alcannin (§ 335), or asphalt (§ 334A) may be employed. For ordinary demonstrations the blue is the best.

345. As the chloroform and turpentine used in these fluids is apt to destroy the cement of the fastenings of the syringe, they may be injected by an apparatus made of a glass tube drawn to a fine point attached to a piece of caoutchouc tubing, which is filled with the injection mass, and closed by a ligature at the other end. On pressing the elastic tube with the finger the fluid is expelled.

346. **Injection of Bile Ducts.**—Inject the portal vein

with carmine and gelatine. Previous to this, open the common bile duct, so that the distension of the blood-vessels may press as much of the bile out of the ducts as possible. Then inject the bile capillaries from the common bile duct with an aqueous solution of soluble Prussian blue. It is not necessary, however, to previously inject the veins. The liver of the rabbit is well adapted for injecting the bile ducts. A pressure of from 0·8 to 1 inch mercury is usually sufficient for the ducts, but it may require to be gradually increased 1·6 inch. The fluid being driven against the bile contained in the liver, an injection of the whole liver must not be attempted, but the injection should be discontinued as soon as a few lobules on the surface of the liver have become distinctly blue. After injection, place the liver in cold methylated spirit. Mount the sections in dammar.

METHODS OF PRESERVING THE TISSUES.

347. **The Dry Method.**—Hair, and sections of dried bone and tooth, are sometimes mounted in air. The difference between the refractive powers of the tissues and the air is, however, so great that the outlines are extremely dark. They show better when mounted in some more refractive medium than air, *e.g.* glycerine, glycerine jelly, or dammar. When a tissue is mounted in air, a ring of dammar or white zinc cement rather smaller than the cover-glass is made on the slide, and allowed to nearly dry. The tissue is then placed within the dammar *cell* (*a*, Fig. 62) and the cover-glass placed over all and pressed upon the dammar, to which it adheres. If the portion of tissue be thick, a glass cell is used (*b*, Fig. 62).

347A. **Dammar and Canada Balsam.**—*a.* All water must be removed from the tissue by drying or by immersion, first in rectified spirit and then in absolute alcohol. The alcohol is driven away by floating the tissue upon oil of cloves, or turpentine, in a watch glass or on a slide, and then the balsam of dammar is added, and the cover-glass put on (see § 152).

b. If Canada balsam be employed, prepare it as follows:—

Place some pure Canada balsam in a saucer or other shallow vessel; cover the vessel with bibulous paper to exclude dust; dry it in an oven at a temperature not above 65° C., until, when it cools, *it becomes as hard as ice;* dissolve this crystalline balsam in benzole. The old-fashioned method of mounting things in balsam was so troublesome, that one avoided it as much as possible. Undried balsam was taken and rendered quite liquid by heat. The slide, the cover-glass, and the tissue to be mounted, all required to be heated. It was difficult to get rid of air bubbles and to avoid over-heating the tissue. But by using a limpid solution of *perfectly dried* balsam, all difficulty disappears.

c. Dammar is now much employed as a substitute for Canada balsam. It renders the tissues more transparent than balsam. The following is an excellent method for preparing the dammar fluid:—Dissolve ½ oz. dammar resin and ½ oz. gum mastic in 3 oz. benzole, and filter. If rapid filtration be desired, use twice as much benzole, and then evaporate the fluid to half its bulk. Chloroform and turpentine are by some recommended as solvents of these resins, but the dammar fluid so made is turbid. If, however, it be prepared as above recommended, it is perfectly clear. Balsam or dammar is suitable for mounting unsoftened bone and tooth, hair, brain, and spinal cord, and indeed most tissues that have been hardened in alcohol or chromic acid, which require to have their transparency much increased. It renders softened bone and tooth too transparent. Speaking generally, it is *more suitable for mounting stained than unstained tissues.* The latter are often rendered too transparent. The tissues very perfectly preserved in either of these substances, because all water is removed.

348. **Glycerine** is not adapted for white fibrous tissue and blood-vessels, unless they have been hardened in chromic acid. Otherwise it causes the white fibres to swell up and lose their normal features, but it is well suited for tissues that are to be examined by very high powers. Liver, lung, alimentary canal, and skin, after having been hardened in chromic acid or alcohol, show better in glycerine than in balsam or dammar, unless they have been stained with car-

mine or logwood. It is suitable for mounting tissues that have beeen stained with silver nitrate or gold chloride. For tissues containing vessels injected with Prussian blue, use two drops H Cl in 1 oz. glycerine, to preserve the colour. It is somewhat difficult to mount things in glycerine. Air bubbles are difficult to eliminate, and if the glycerine pass outside the cover-glass, it is difficult to get rid of it. It can be removed most readily by sucking it up with a fine glass pipette, Fig. 45). It is often necessary, however, to transfer the preparation to a new slide, on which a smaller drop of glycerine has been placed. The faults of glycerine are—1, a tendency to render the tissues too transparent; 2, a difficulty in getting rid of superfluous fluid; 3, a difficulty in getting rid of air bubbles.

349. **Farrants' Solution** is an excellent substitute for glycerine in many instances indicated in the preceding demonstrations, because of its feebler tendency to render the tissues transparent. It consists of "equal parts of gum-arabic, glycerine, and a saturated solution of arsenious acid" (*Frey*). Owing to its containing gum it becomes hard, so that the cover-glass becomes fixed at its margin. In mounting preparations with this fluid, the covered object is allowed to lie for a day before the varnish is applied, so that the cover may be fixed and thereby prevented from shifting during varnishing, as so often happens with glycerine.

350. **Glycerine Jelly** is a good preservative agent for many tissues, such as lung, blood-vessels, tooth, bone, cochlea, etc. The tissues must be steeped in weak spirit (§ 352) previous to mounting. They cannot be transferred from glycerine to glycerine jelly directly. Fungi are very apt to grow in some kinds of glycerine jelly that are sold. Rimmington's glycerine jelly is the best. Its composition is unknown, but it appears to contain glycerine, gelatine or isinglass, and carbolic acid.

351. **Potassium Acetate.**—A saturated solution of this substance has long been used by botanists. It is the best medium for osmic acid preparations (*Schultze*). Glycerine renders these too transparent. The fluid may be prepared

thus :—Dissolve 56 grammes potassium acetate in 28CC hot water. When cold, add 2CC camphorated spirit, and filter.

352. **Weak Spirit**—consisting of rectified spirit 1 part, distilled water 3 parts—was formerly much employed. Muscle, connective tissue, and blood-vessels, can be well preserved in it for a time, but sooner or later they become granular and useless.

352A. **Rectified Spirit** may be used for mounting softened bone and tooth. Glycerine renders these organs too transparent if they are unstained.

353. **Naphtha and Creosote fluid** is useful for preserving urinary casts (§ 222).

354. **Cells for Microscopic Objects.**—Cells are made of tissue paper, tinfoil, cardboard, dammar varnish, or white zinc cement, or of glass. A paper cell is made by cutting with a punch a paper circle the size of the cover-glass. A hole is then cut in the centre with a punch of smaller size. A cell of dammar or white zinc varnish (*a*, Fig. 62) is made

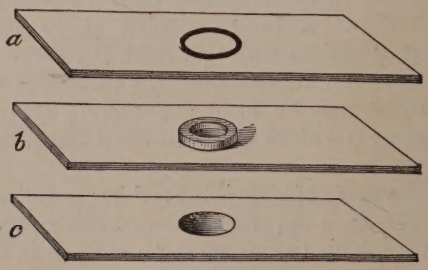

FIG. 62.—Cells.

by tracing a ring of the substance on the slide with the aid of Shadbolt's turn-table (Fig. 63), and then allowing it to dry. A glass cell (*b*, Fig. 62) usually consists of a section of a glass tube cemented to a slide with marine glue. Sometimes a sunk glass cell (*c*) is employed. It is merely a depression ground in the glass. Delicate tissues that are apt to be spoilt by the weight of the cover-glass should be placed in a cell of tissue paper or gold-size.

355. **Turn-table.**—The dammar or white zinc cells above mentioned are made with the aid of Shadbolt's turn-table (Fig. 63), consisting of a rotary brass disc with a series of concentric lines around its centre. There are clips for fixing a slide, and a wooden board for supporting the whole. The slide is centred and fixed by the clips. A brush dipped in the varnish is held over one of the rings, and the table turned rapidly. The apparatus is also used for putting a ring of varnish round the margin of the cover-glass in sealing up a preparation.

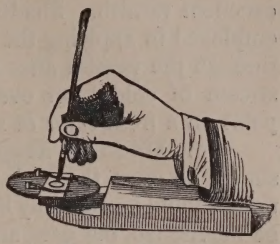

FIG. 63.—Turn-table.

356. **The spreading out of Sections.**—In dealing with large sections or with membranes there is often considerable difficulty in spreading them out. This difficulty may, however, be readily overcome by floating them on to the slide by the method described in § 87.

357. **Mounting-clip.**—In the case of sections that can bear some pressure, and which are difficult to keep perfectly flat, a mounting-clip may be applied until the cover-glass is fixed by glycerine jelly, dammar, or some cement (Fig. 64). It consists of a brass wire bent so as to form a spring. The end that is applied to the cover-glass is covered with leather. In the case of bone, tooth, or cartilage, it may be used, but the section is apt to be spoilt unless the spring be feeble, and, indeed, in most cases no such thing need be used.

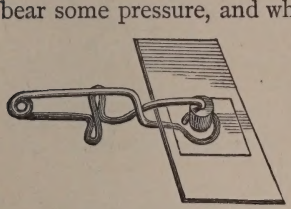

FIG. 64.—Mounting-clip.

358. **Cements.**—When the object is mounted in Canada balsam or dammar, no cement is required; but if any other preservative substance be employed, even glycerine jelly—for it is hygroscopic—the margin of the cover-glass must be varnished. Dry the slide with bibulous paper, then paint on a layer of *gold-size*, allow it to dry, and lastly

cover this with white zinc cement. The latter gives an elegant finish to the preparation, and is at the same time an excellent varnish. Shadbolt's turn-table (Fig. 63) is often employed in applying the varnish. Glycerine is apt to ooze through the varnish after a time; when this happens, run a stream of cold water over the preparation, then dry and paint on a fresh layer of zinc cement.

359. **Mode of keeping Preparations.**—The slides should lie upon the back, *not* upon the edge. All preparations should be carefully labelled. The things shown should be mentioned, the date, and the name of the mounting fluid ought also to be stated. *Never keep a bad preparation, and don't attempt to preserve everything.*

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